Growth of Larval and Juvenile Newts

Thesis

Growth of Larval and Juvenile Newts

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Abstract

In smooth newts (*Triturus vulgaris*), adult body size is determined by mainly by growth during the pre-maturity stages. Adult body size is frequently related to reproductive success and hence fitness. This project examined factors affecting growth during the pre-maturity stages.

Chapter 2 reviews the literature on the effects of adult body size on reproductive success in urodeles with particular reference to newts of the *Triturus* genus. This review identifies the need to experimentally test and further quantify body size effects. Two such studies are reported in Chapter 3.

Chapter 3 examines the relationship between female body size and fecundity in female smooth newts and also examines short-term mating capacity in males. Female body size is strongly related to fecundity but there is only a weak relationship between male body size and the number of spermatophores deposited.

Chapter 4 describes the pattern of growth of newt larvae (*T. alpestris*, *T. cristatus* and *T. vulgaris*) grown in the laboratory. Variation in larval period explains most of the body size variation at transformation. Chapters 5, 6 and 7 report studies investigating the effects of temperature, food availability, larval density, maternal effects, population effects and timing of oviposition on the growth of *T. vittatus* and *T. vulgaris* larvae in the laboratory and semi-natural conditions.

Chapter 8 investigates growth during the eft stages of *T. cristatus* and *T. vulgaris*. The rank ordering of body sizes of individuals at transformation is preserved during the eft stage in *T. vulgaris* and *T. cristatus*, and to adulthood in *T. cristatus*.

Variability between growth strategies of individual newts may have been naturally selected as a response to larval life in a variable and unpredictable environment.

Chapter 1
Introduction

In adult smooth newts (*Triturus vulgaris vulgaris*) it has been suspected that reproductive success (R.S.) is highly correlated with body size in both males (Verrell and Francillon 1986) and females (Bell 1977, Verrell and Francillon 1986). Newts show indeterminate growth; ie. individuals continue to grow throughout their lives. Hence it has been assumed that a big newt was simply an old newt and age could explain the large variances observed in adult body size. Adopting this rationale, Bell (1977) used body size as a means of ageing smooth newts. However, both long term field studies and the advent of skeletochronology (a technique of ageing seasonally growing ectotherms by examining growth rings in bone tissue) refute the validity of this assumption in relation to urodeles (Halliday and Verrell 1988).

Within a population of *Notophthalmus viridescens*, Gill (1978) found that the sizes of old adult and first time breeders overlapped. Ovaska and Gregory (1989) in a study of *Plethodon vehiculum* found that first year juveniles formed a discrete size class, but second year individuals tended to merge with older salamanders. Results from field studies of this nature show that body size does not segregate urodeles into discrete age classes.

An implicit assumption of the use of body size for ageing urodeles is that individuals of a certain age class should exhibit similar growth rates. However, Hagström (1980) in a skeletochronological examination of some newts that he captured in a previous study (Hagström 1977) showed that growth in *T. cristatus* and *T. vulgaris* is not related to age. Other skeletochoronological studies, of newts in particular, (*Triturus vulgaris* [Verrell and Francillon 1986], *T. vulgaris* and *T. cristatus* [Hagström 1977], *T. cristatus* and *T. marmoratus* [Francillon-Viellot et al. 1990]) and of amphibian species in general, have shown that body size is only weakly correlated with age and that there is large variance in body size at any particular age. Thus, records of growth of urodeles in the field and skeletochronological studies lend support to the rejection of the notions that body size can

be used to age urodeles and that natural selection for larger size reflects selection of older individuals with survival abilities proven by age (review in Halliday and Verrell 1988).

It is also known that growth after the attainment of sexual maturity is slow. Bell (1977) recorded annual growth in snout-vent length (SVL) of *T. vulgaris* as 1.05 mm from seven individuals that he was able to recognise on recapture. Hagström (1977) identified individual *T. vulgaris* and *T. cristatus* by photographing the belly patterns. Recapture of the same individuals over successive years showed that adult growth was slight or even zero (*T. cristatus* means from 0.5-0.6 mm per year, *T. vulgaris* means 1.0-1.2 mm per year). Glandt (1981) also recorded zero and negative growth rates for some individuals in a free ranging population of *T. cristatus*. Skeletochronological studies can also be used to deduce growth rates, by measuring the gradient of a linear regression of body size on age. This method produces results similar to those obtained by recapture of known individuals. For *T. cristatus* means of 0.8-1.3 mm per year and for *T. vulgaris* means of 0.1-0.9 mm per year have been recorded by Hagström (1977). For *T. vulgaris* 1.5 mm per year was recorded by Verrell and Francillon (1986). For *T. cristatus* 0.7 mm per year and *T. marmoratus* 1.3 mm per year were recorded by Francillon-Viellot et al. (1990). Since adult growth is so slight, newts that are small when they first reproduce would be expected to remain small in relation to the same-age individuals, and have a lower lifetime reproductive success, compared with those that attain large body size by the time that they first breed. It thus appears that lifetime reproductive success may be determined primarily by growth in the larval and juvenile stages of the life cycle (Halliday [1987] describes this effect for male smooth newts, and see Halliday and Verrell [1988]) and hence it follows that selection for large adult body size may well be exerting selection pressure on growth during these stages (Trivers 1976). Juvenile growth and body size at maturity has been shown to have fitness consequences over a wide range of species, eg. *Daphnia pulicara* (Tessier and Consolatti 1989), *Drosophila melanogaster* Roff (1981) and *Corvus corone corone* (Richner et al. 1989) which suggests that natural selection acting on pre-maturity growth may be a general phenomenom throughout much of the

animal kingdom.

1.1 Description of Life Cycle and Growth of Newts Amphibia exhibit complex life cycles: during the life of an individual, there is transformation from one form to another, accompanied by a change in ecological niche (Wilbur 1980). In amphibia, this transformation usually consists of a change from an aquatic larva, to a terrestrial juvenile or adult. There is much variation on this pattern both between and within species. Typically, in Great Britain, the life cycle of the smooth newt (*Triturus vulgaris vulgaris*) proceeds as follows. In the spring, adults congregate in still bodies of water, where they court and females subsequently oviposit. The ova hatch out as larvae, which are the fastest growing stage of the life cycle. The larval stage of amphibian life cycles is often presumed to be an adaptation to exploit an abundant food source in order to maximize growth rate (Wilbur and Collins 1973, Wilbur 1980).

The larvae attain transformation in late summer or autumn and then leave the water. At this point SVL measures about 19 mm (Verrell 1985). These metamorphs assume a terrestrial life for roughly the next one and a half to two and a half years, during which time they grow slowly, so that at maturity the SVL measures about 44 mm (from Verrell and Francillon 1986). This terrestrial phase between transformation from the larval form and sexual maturity is termed the eft stage. Two theories have been proposed to explain the adaptive significance of this stage:

1. Healy (1974). The eft stage is an adaptation that allows exploitation of a relatively benign terrestrial environment and to avoid competition for food in ponds.
2. Gill (1978). This stage is a mechanism to colonize new ponds in an unstable environment. i.e: In an environment where ponds deteriorate with time and new ponds are formed. Wilbur (1980) also regards the terrestrial stage of complex life cycles as being a dispersal stage.

There is also a third alternative. It is possible that the eft stage is an adaptation to avoid mortality due to ponds freezing during the winter. Such situations occur in

populations of *Triturus alpestris* at high altitudes. Larvae that do not transform before the onset of winter are killed as ponds freeze through (Helmut Faber and Robert Schabetsberger, pers. comm.).

The above theories are not mutually exclusive, but Healy's theory seems unlikely to be true in practice, since he himself notes (1973) that aquatic juvenile *Notophthalmus viridescens* grow faster and mature earlier than the terrestrial eft form, which suggests that the pond is in fact a better growth environment than that experienced by terrestrial juveniles.

When considering colonization of new ponds, Bell (1977) tentatively suggested that movements of efts may be responsible, but he thought that such migrations were infrequent. Frazer (1989 p. 111) also considers that smooth newt efts are the colonizing stage of the life cycle. A similar situation seems likely to exist among other species of *Triturus*. In a skeletochronological study of *Triturus marmoratus* (Caetano et al. 1985), the lack of juveniles found in and around breeding ponds suggests that they had indeed dispersed.

Despite this emphasis on colonization, it should be noted that a large amount of growth can actually occur in the juvenile, terrestrial phase of amphibian life cycles. Werner (1986) notes that in North American anurans at least 80%, and usually more than 90%, of adult body weight is accounted for by growth during the juvenile stage. Weights of local smooth newt metamorphs have not been recorded, but it is possible to estimate what proportion of adult body weight is attributable to growth during the eft stage. Snout-vent length at transformation is 19 mm (Verrell 1985) and this increases to 44 mm at maturity (Verrell and Francillon 1986). If it is assumed that body weight increases as a cubic function of snout vent length, then it is possible to calculate an approximate value of weight change over the eft stage:

$$19^3 = 6859$$

$$44^3 = 85184$$

Therefore metamorph weight as a percentage of adult weight is:

$$6859/85184 = 0.0805 = 8\%$$

A metamorphic smooth newt should weigh about 8% of adult body weight, which means that another 92% of adult weight is accounted for by eft growth. So, as noted by Werner, much growth occurs during the terrestrial juvenile stage.

Once adult, smooth newts return to the water to breed. Smooth newts are iteroparous, and Verrell and Francillon's (1986) skeletochronological work suggests that the lifespan of newts in southern England may encompass four or five breeding seasons. Adult life is divided into aquatic and terrestrial phases. Aquatic phases are necessary for reproduction whilst the remainder of the year is spent in a terrestrial habitat. However, gonadal activity occurs outside the mating period, so that development of ova and spermatozoa are dissociated (*sensu* Crews 1987) from the period of courtship and oviposition (Verrell et al. 1986).

Although growth of amphibians is usually regarded as being indeterminate, growth of adult smooth newts seems to be very slight (see above). It also seems that most adult growth probably occurs during the aquatic phases of adult life. Verrell and Halliday (1985) found that the longer an individual remained in a pond over the breeding season, the more weight it gained, and Verrell (1987) noted a 1.1-1.5 mm increase in SVL over this period in some individuals. This size increase is of the same order of magnitude as the total annual growth of adult smooth newts (see above).

There is no longitudinal study of growth of *T. vulgaris*, tracing larvae through to adulthood, but it is possible to reconstruct the pattern of growth throughout the life cycle using data from Verrell (1985) and Verrell and Francillon (1986) (see Fig. 1.1).

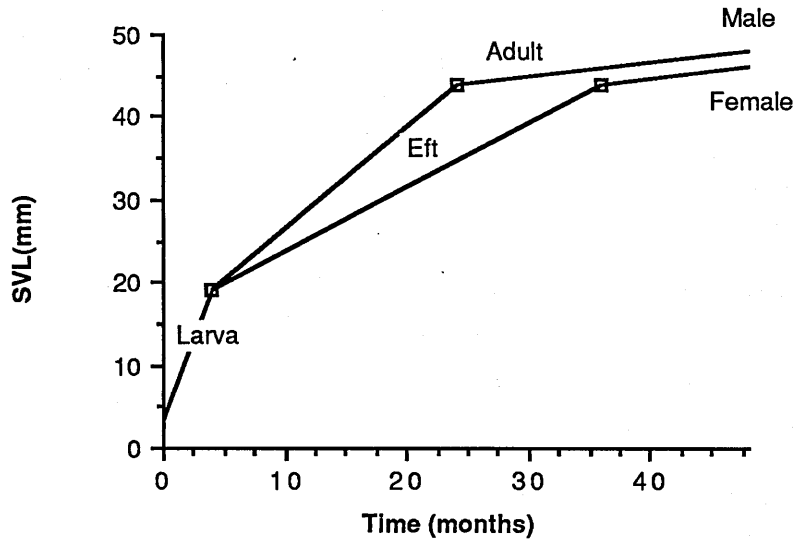


Fig. 1.1. A representation of growth during the life of *T. vulgaris*.

Information used to generate the above scheme:

Larval period of 4 months estimated from Verrell (1985).

Median SVL of metamorphs (19 mm) from Verrell (1985).

Males sexually mature at 2 years from Verrell and Francillon (1986).

Females sexually mature at 3 years from Verrell and Francillon (1986).

Growth after sexual maturity (1.5 mm/year) from Verrell and Francillon (1986).

1.2 Variance in Adult Body Size To demonstrate the variation in adult body size within a population, size-frequency distributions from a population of smooth newts are shown in Figs. 1.2 and 1.3. These data were obtained from adult newts which were captured at a site in Milton Keynes (Great Linford) as they migrated towards a breeding pond. All newts were anaesthetized using MS-222 (Sandoz), SVL measured to the nearest 0.5 mm, and weight to the nearest 0.01g was recorded. For details of measuring adult body size, see Chapter 3. The body size data collected are summarized in Table 1.1.

		Mean	Skewness	Range	n
Males	SVL (mm)	46.5	0.110	39-53	58
	Wt. (g)	2.46	0.511	1.55-3.58	58
Females	SVL (mm)	45.4	0.127	39-52	47
	Wt. (g)	2.36	0.977	1.31-3.93	47

Table 1.1. To show the variation in body size of a sample of smooth newts collected from Great Linford, Milton Keynes in 1988.

From Table 1.1 and Figs. 1.2 and 1.3 it can be seen that there is considerable variation in adult body size. The largest male is over twice as heavy as the smallest, whilst female body weight increases by a factor of three over the size range of the population. From Figs. 1.2 and 1.3 it can be seen that, in both sexes, SVL appears to have a fairly normal distribution and weight is positively skewed. Harrison et al (1984) measured total lengths of smooth newts from a population in Mid-Wales and also found normally distributed size-frequencies in both sexes. If large body size was being selected, then it might be expected that one would observe a negatively skewed data set. However, there are various reasons why the data presented are fairly typical. Body weights of organisms

are typically skewed this way (Uchmanski 1985). The mechanism that produces this pattern is debated: Uchmanski (1985) proposes that this is due to competitive interactions between individuals. However, it has also been suggested that non-interacting individuals can produce a positively skewed weight distribution as part of the typical growth process (Koyama and Kira 1956). This assumes that body weights of a cohort are initially normally distributed and will grow according to normally distributed relative growth rates, which produce a positively skewed weight distribution in later life. However, these explanations of the frequently observed positive skew in weights explain variance in body size for a single-aged cohort, and it is unlikely that this is true of newt populations which consist of adults of many ages. Hence, the positive skew in body weights could be due to the smaller numbers of older (and on average) larger animals in the population, relative to younger cohorts.

Since so much variation in adult body size seems to be generated during pre-maturity growth, and this variation affects R.S., a project to examine factors affecting growth during this stage of the life cycle warranted further investigation. Natural selection is a process that is driven by differential survival and reproduction of individuals, and it seems that juvenile growth may well be a major factor contributing to differential reproduction within a breeding population. High pre-maturity growth rates should produce large, reproductively successful adults, that should pass on genes for high juvenile growth rate to their offspring. These offspring should outnumber the offspring of smaller adults, with poor juvenile growth. The smooth newt (*Triturus vulgaris vulgaris*) was chosen as the focal species of the present project, because it is a common vertebrate, found locally, and its reproductive ecology is the most thoroughly studied within the *Triturus* genus.

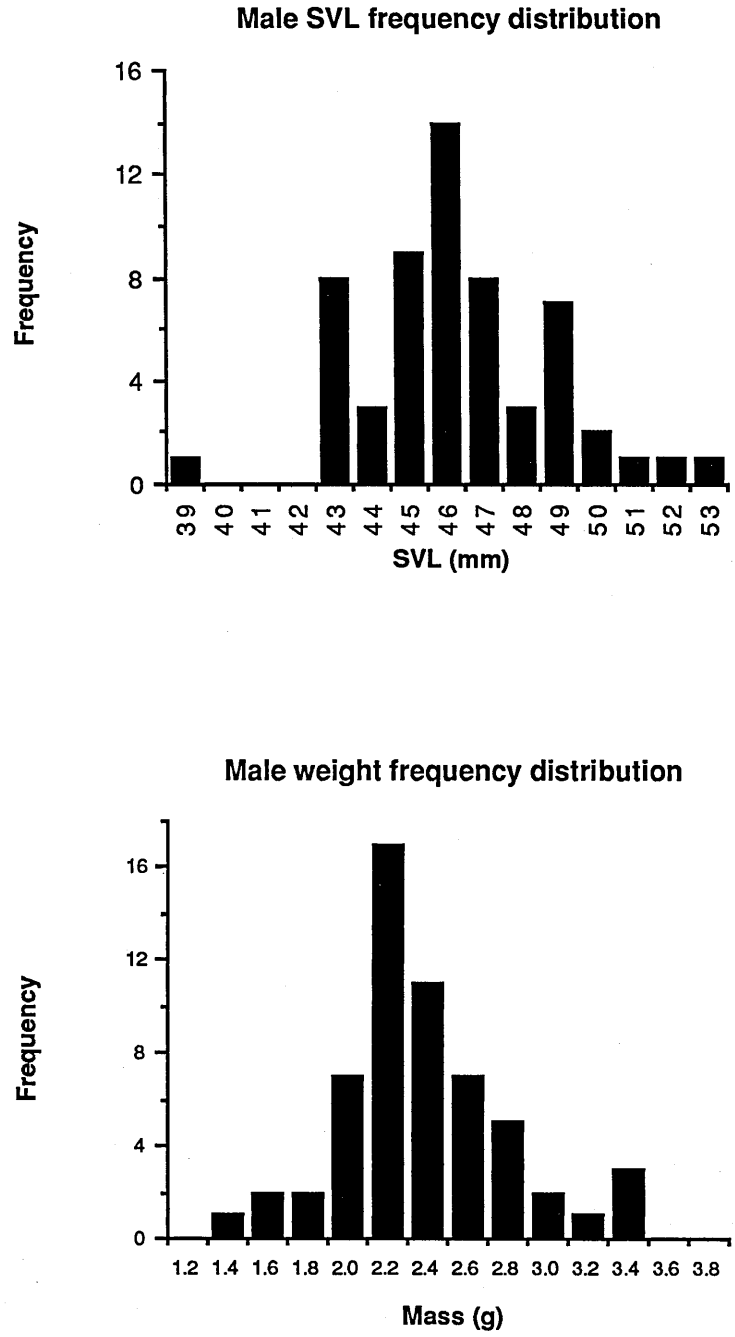


Fig. 1.2. Histograms to show size-frequency distribution of a sample of 58 male smooth newts (*Triturus vulgaris*) from a single population. Upper graph shows frequency distributions of SVL. SVL of 39 mm refers to all newts measured as 39 or 39.5 (newts were measured to the nearest 0.5 mm) etc. and weight of 1.2 g refers to all newts weighing from 1.20-1.39 g inclusively, etc.

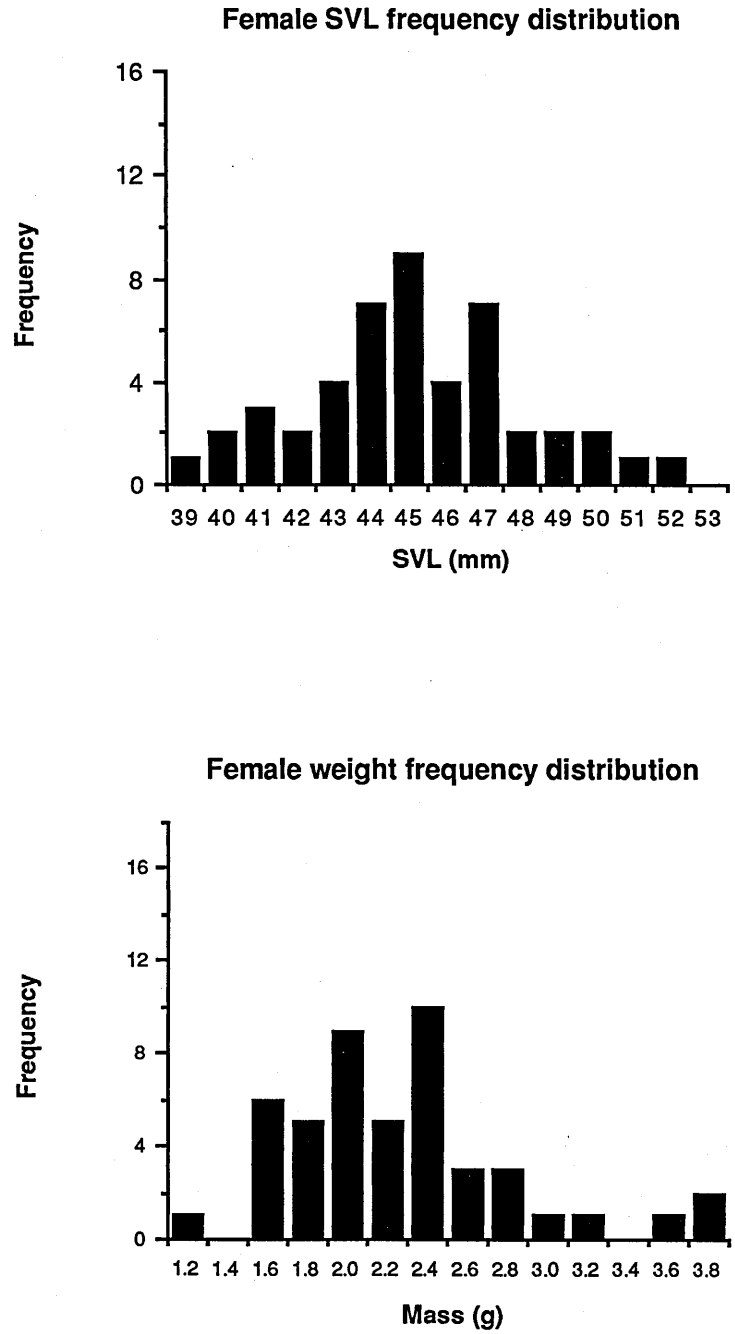


Fig. 1.3. Histograms to show size-frequency distribution of a sample of 47 female smooth newts (*Triturus vulgaris*) from a single population. Upper graph shows frequency distributions of SVL. SVL of 39 mm refers to all newts measured as 39 and 39.5 mm (newts were measured to the nearest 0.5 mm) etc. and weight of 1.2 g refers to all newts weighing from 1.20-1.39 g inclusively, etc.

Chapter 2

Body Size and Individual Reproductive Success

Within the field of animal behaviour body size is frequently used as an index of fitness, usually due to the greater reproductive success (R.S.) of larger individuals. This chapter addresses two questions:

1. Does the current literature support the notion that body size is positively correlated with R.S. (within a species or population) among the urodeles in general and *Triturus* in particular?
2. How, if at all, does the size of an adult newt affect its reproductive success and hence fitness?

With respect to reproductive success, body size could be potentially subject to natural selection, in females, via differential fecundity and/or male mate choice. In males, natural selection may act on reproductive success through competition for access to females and/or female mate choice. There must also be other, non-reproductive, selection factors acting on body size. For example, it might be expected that small urodeles would be subject to greater water loss via dehydration, due to their large surface area to volume ratios; and a between-species study of plethodontid salamanders shows that this is indeed the case (Spotila 1972). The same surface area to volume effect is also responsible for the higher oxygen exchange per unit body weight of smaller sirenid salamanders (Ultsch 1976). However such environmental selection factors are beyond the scope of the present thesis. The following reviews (Sections 2.1, 2.2 and 2.3) confine themselves to the question of whether body size and reproductive success are related in urodeles.

2.1 Female Fecundity

Female fecundity is closely linked to Darwinian fitness, since an individual that is able to produce more viable offspring during its lifetime than another conspecific, will be represented by a larger fraction of the gene pool in the future generations. So individual

clutch mass can be used as an index of fitness. Clutch mass being the total investment in ova (a product of ovum size and number). The question to bear in mind is whether clutch mass is a function of body size. If a positive correlation between female body size and fecundity exists, and providing that body size is in fact inherited, then larger body size will be selected. Large females will produce more offspring than small females and these offspring will mature as large adults. The degree of heritability of body size is not clear, since environment may affect size to such an extent that heredity becomes an insignificant factor. At this point it is also relevant to consider the argument that if a character is strongly linked to fitness, then heritability of that character may be expected to be low because all the additive genetic variance should have been removed from the population by natural selection (Falconer 1960).

Clutch mass is related to body size between species in teleostean fish (Duarte and Alcarez 1989), amphibia (Crump and Kaplan 1979, Kaplan and Salthe 1979) and lizards (Tinkle et al 1970). Reiss (1989) provides a good review of work that shows this relationship. It has been proposed that body size imposes physical limits to clutch size in amphibia (Crump and Kaplan 1979, Kaplan and Salthe 1979). In fish (Wootton 1990) and squamate reptiles (Cooper et al. 1990) there are locomotory costs to being gravid, which may also constrain clutch size in a manner related to body size.

Within species there is also evidence that a positive relationship between female body size and clutch mass exists. Table 2.1. lists such work results for urodeles. However, it should be noted that a positive correlation, within a species, does not necessarily prove that clutch mass is constrained by body size. The observed relationship between body size and clutch size does not infer causality. The body size constraint on clutch size must be treated as one of four possible alternatives.

The second alternative is that the pattern of resource allocation changes as individuals grow. In species that exhibit indeterminate growth and iteroparous reproduction, such as many fish (Weatherley and Gill 1987), smaller females may invest more resources in somatic growth than in reproductive growth, whereas larger females may reverse this

pattern of resource investment. It is already known that adult growth is often shown to be inversely related to body size (eg. *Cryptobranchus alleganiensis* [Taber et al. 1975], *Triturus vulgaris* [Verrell 1987], *Rana temporaria* [Ryser 1988]). If urodeles are indeed investing less in growth and more in reproduction as they grow, then this would also produce a positive relationship between female body size and clutch size. This is also the type of explanation of the relationship between female body size and clutch size favoured by Reiss (1989).

The third alternative is that small females may be small for the very reason that they are poor at recruiting resources. Individuals that suffer lower resource input (due to chance or inherited effects) may not only have less resources available for growth, but also less available for allocation to reproduction, in which case body size would indicate past growth history and would also be related to clutch mass, but not in a causal manner.

Fourthly, Williams (1966) noted a life history trade-off between sequential reproductive efforts. Like Lack (1954) he proposed that the amount of energy that iteroparous species should invest in reproduction each year must be a compromise between maximizing clutch mass in that year `and allowing the female to survive to reproduce the next year. Hence there is a theoretical optimum energy investment. As an individual draws nearer to the end of its expected life, and hence becomes older, the optimum reproductive effort should increase. So, in species with indeterminate growth, a positive correlation between body size and reproductive effort could exist because larger females tend to be older and therefore invest more in the reproductive effort in question.

All four of the above hypotheses, namely, body size physically restricting clutch size, a shift in resource allocation from growth to reproduction as a female grows, absolute energy available for growth and reproduction varying between females, and increasing reproductive effort as a function of age would yield a positive relationship between female body size and clutch size.

Species	Female Parameter	Clutch parameter	Source
Triturus vulgaris	Weight	Ovarian oocytes	Verrell 1986b
Triturus vulgaris	Weight	Number of ova deposited on one insemination	Verrell 1986b
Triturus vulgaris	SVL	Ovarian oocytes, ovary size and oocyte diameter	Verrell and Francillon 1986
Necturus punctatus	SVL	Ovarian oocytes	Meffe and Sheldon 1987
Notopthalmus viridescens	SVL	Ovarian oocytes	Verrell 1982a
Ambystoma opacum	SVL	Number of ova produced	Walls and Altig 1986
Ambystoma texanum	SVL	Ovarian oocytes	Petranka 1984
Hynobius tsuensis	Wt.	Wt. ova and jelly after induced spawning	Kuramoto 1978
Onychodactylus japonicus	Wt.	Wt. oocytes in ovary	Kuramoto 1978
Cynops p. pyrrhogaster	Wt.	Wt. ova and jelly after spawning	Kuramoto 1978
Desmognathus fuscus	SVL	Number of eggs in clutch	Hom 1987
Desmognathus ochrophaeus	SVL	Ovarian follicle count	Tilley 1973
Gyrinophilus porphyriticus	SVL	Ovarian oocytes	Bruce 1969
Pseudotriton ruber	SVL	Ovarian oocytes	Bruce 1969
Pseudotriton montanus	SVL	Ovarian oocytes	Bruce 1969
Ambystoma tigrinum	Body volume	Clutch volume	Kaplan and Salthe 1979
Ambystoma maculatum	Body volume	Clutch volume	Kaplan and Salthe 1979
Ambystoma opacum	Body volume	Clutch volume	Kaplan and Salthe 1979
Plethodon glutinosus	SVL	Ovarian ooctye count	Semlitsch 1980
Ambystoma annulatum	SVL	Ovarian ooctye count	Hutcherson et al. 1989
Bolitoglossa rostrata	SVL	Ovarian count and number of ova deposited	Houck 1979

Table 2.1 Studies that have found a positive relationship between female body size and some parameter of clutch size in urodeles.

Species	Female Parameter	Clutch parameter	Source
Triturus cristatus	SVL	Ovarian oocytes	Hagström 1980
Triturus vulgaris	SVL	Ovarian oocytes	Hagström 1980
Ambystoma maculatum	SVL	Ovarian oocytes	Shoop 1974
Desmognathus monticola	SVL	Ovarian follicles	Tilley 1968
Plethodon larselli	SVL	Ovarian oocytes	Herrington and Larsen 1987
Plethodon cinereus	Not given	Not given	Blanchard 1928
Plethodon cinereus	SVL	Ovarian follicles	Nagel 1977
Plethodon cinereus	SVL	Oovarian oocytes	Fraser 1980

Table 2.2 Studies that have failed to show a positive relationship between female body size and clutch size.

Body size and fecundity in Triturus There are little data on body size and clutch mass within this genus, except for *T. vulgaris* (see below). Data collected from *T. alpestris* and *T. marmoratus* are presented in Fig. 2.1. The *T. alpestris* were one year old, laboratory-reared individuals that all died prior to their first breeding season. Reasons for death are not known. The *T. marmoratus* were from various sources (laboratory-reared and wild-caught) and hence were of various ages. These also died during a winter, and hence immediately prior to reproduction. All females were preserved in 4% formalin. Later they were towel dried and weighed. The ovaries were then dissected out and also towel-dried and weighed. Even with the small sample sizes used here (*T. alpestris* n = 5, *T. marmoratus* n = 6) there is a strong positive relationship between body size (mass) and ovary mass. It is expected that larger sample sizes using other *Triturus* species would yield similar results.

Body size and fecundity in Triturus vulgaris Size-fecundity relationships have been found for female *T. vulgaris*. Bell (1977) showed that larger, or as he assumed, 'older' females contain more yolked oocytes. Verrell (1986a) showed that body size (weight) and number of yolked oocytes in the ovaries are positively correlated. The number of yolked ova ranged from 100-500. He also recorded a significant positive correlation between female weight and ova produced after one insemination (that is that the female only picked up one spermatophore) but the range in this case was 25-80 ova; possibly a reflection of the number actually fertilized rather than true clutch size. Verrell and Francillon (1986) examining females at the beginning of the breeding season found that SVL correlates positively with ovary size, number of yolked oocytes and mean oocyte diameter. This latter relationship may have important fitness consequences (see below and Section 5.4).

However it should be noted that the size-fecundity relationship has not held true for all examinations of urodeles. Hagström (1980) counted the eggs in the ovaries of female *Triturus cristatus* and *T. vulgaris*, prior to breeding, and found that there was no

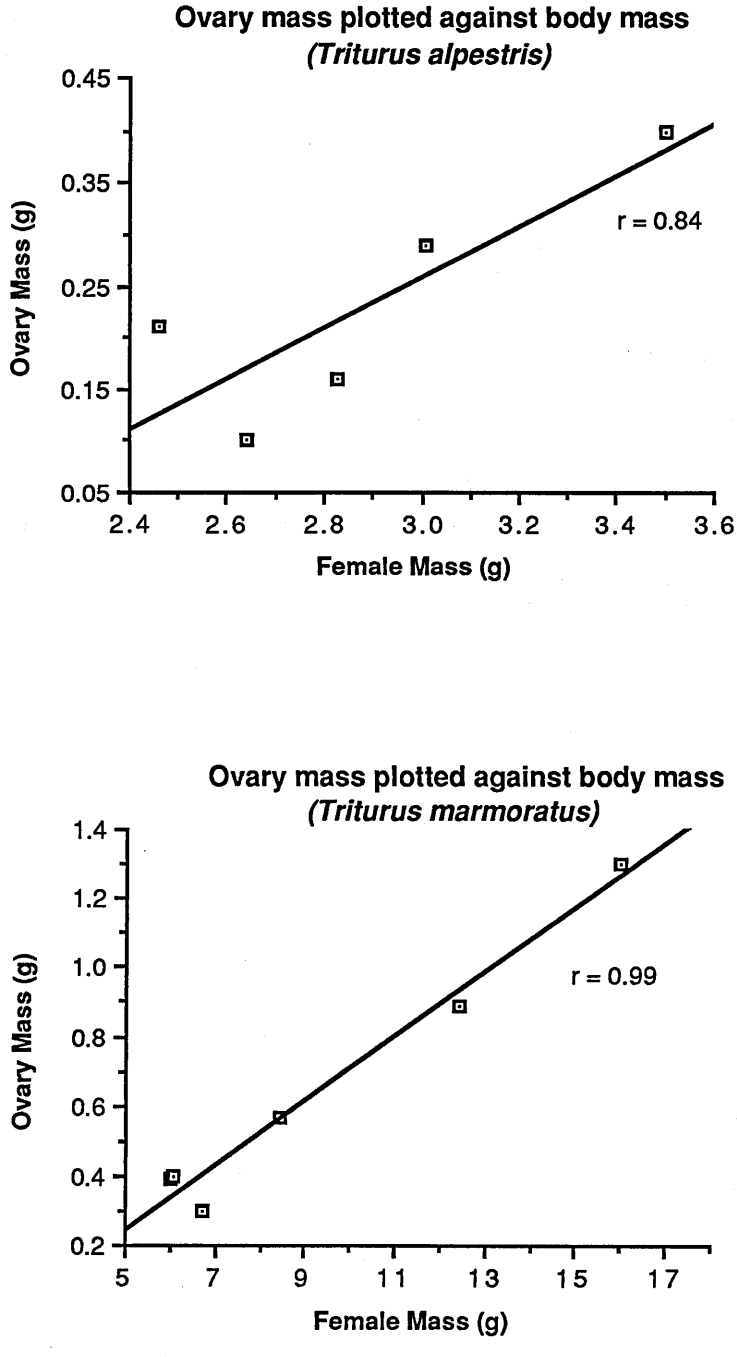


Fig. 2.1. Mass of ovary plotted against mass of female for two species, *Triturus alpestris* and *Triturus marmoratus*.

relationship between either the numbers of yolked eggs or total eggs and female body size. He also recorded smaller numbers of yolked ova (71-282) in *T. vulgaris* than Verrell (1986a). Fraser (1980) cites two other examples in which clutch size parameters are not related to body size in *Plethodon cinereus*. To these examples, I add five other species and Fraser's own experimental results in Table 2.2.

Possible reasons for the lack of relationship between body size and fecundity Fecundity may be affected by other non-size related factors. Differences in foraging ability or 'resource accruing abilities' could well affect clutch size. Resources accrued may or may not be related to body size. Alternatively, resource allocation may vary between individuals, such that one female may invest more in somatic growth rather than in reproductive growth (oocyte development). Bruce (1969) postulated that there may be a genetic component involved in determining clutch size and this may generate variance which is unrelated to female body size. There may also be an element of chance that determines female fecundity and which may mask other effects. For example Ryser (1989) found that reproductive effort of individual female *Rana temporaria* was not inversely related to subsequent growth or survival which suggests that these frogs were not subject to trade-offs between somatic and reproductive growth or between reproductive effort and survival. Ryser suggests that environmental factors must be affecting female R.S. Between years there was no correlation between output of individuals, which again suggests an environmental rather than genetic influence on clutch mass. Although he does not expand on the mechanism of this effect, presumably it is due to differences in quality of the environment experienced by adults during the terrestrial phase, when the frogs are feeding. In the laboratory, manipulation of feeding levels can affect clutch parameters in convict cichlids, *Cichlasoma nigrofasciatum*, (Townshend and Wootton 1984) and fire-bellied toads, *Bombina orientalis*, (Kaplan 1987). However, Fraser's (1980) experimental manipulation of feeding in *Plethodon cinereus* failed to show any detectable trends. Fraser speculated that the nature of the foraging environment of salamanders controls clutch mass, within the constraints of body size. An adult amphibian that feeds

solely during the terrestrial phase (eg. *Rana temporaria*) or during the aquatic and terrestrial phases (eg. *Triturus vulgaris*) may by chance migrate to a poor terrestrial feeding area, and due to the relative immobility of newts, may be subjected to this environment for longer periods than another individual, which by chance, spends the whole terrestrial phase in a productive feeding area. Such chance factors affecting rate of food acquisition could affect resources allocated to reproduction and hence clutch size, in a non-body size related manner, or may simply create 'noise' in any detectable body size-clutch size relationships.

It is also possible that large body size is not always advantageous to a female. For example in harsh environments, where prey items may be scarce and/or opportunities to forage limited, the higher demands of large body size maintenance may preclude the opportunity to sequester resources for reproduction.

Another point which must be considered in a discussion of fecundity in *Triturus* species is that it is by no means clear that female *Triturus* actually oviposit all of the ova that are yolked at the beginning of the year. Hagström (1980) netted females when he assumed that oviposition had ceased, and found that these animals still contained yolked ova in the ovaries. It is always possible that these females had not actually finished oviposition, but Hagström's opinion that not all yolked ova are oviposited each season is given further support by Harrison (1985) who captured females as they were moving away from a pond at the end of the breeding period, and found that they also contained yolked ova. Verrell et al. (1986) examined the ovaries of female *Triturus vulgaris* throughout the year and found that the number of yolked oocytes decreased through the season, suggesting that these oocytes are used up each year. Females that they examined leaving the water in the Autumn did contain yolked oocytes, the interpretation of this being that these were yolked post-nuptially and were not left over from the previous period of oviposition. So, it is not clear whether female *Triturus* oviposit all of their yolked oocytes each year.

One further, potential, reproductive advantage that large females may have over small females is in terms of resources invested in each individual ovum. The relevance of clutch

size to individual female fitness, discussed above, rests on an implicit assumption that large females produce more ova than small females. If reproductive success depended solely on maximizing egg number, and it is accepted that female clutch size is limited in some way, then high fecundity could be assured by dividing the resources allocated to reproduction into many small ova. However, it is quite clear that all animals do not produce uniformly small propagules (units of propagation such as ova or offspring). Variation in offspring size between species is probably dictated by such factors as parental body size and nature of environment. For example, an upper limit to infant size in primates is determined by the size of the birth canal of the female (Leutenegger 1973). However, ovum size in teleostean fish does not seem to be related to adult body size alone, but also to predictability of the larval growth environment (see Duarte and Alcarez 1989). It is also notable that within a species not all propagules are the same size. Variance is also noted between females within a population, or within a single clutch (eg. *Ambystoma opacum*, *A. maculatum*, *A. tigrinum* [Kaplan 1980], *Taricha torosa* [Kaplan 1985], *Hyla crucifer* [Crump 1984], *Bombina orientalis* [Kaplan 1989]) and variance has also been recorded within neonates of a single *Daphnia* clone (Tessier and Consolatti 1989). The basis of this deviation away from maximizing number of propagules may be due to the fact that larger propagules may have some survival advantage over smaller propagules, hence higher parental investment in each propagule is selected. For example in fish, big eggs produce big larvae, with higher survival than larvae from small eggs (Wootton 1990). Laboratory studies of amphibia have also shown the effects of propagule size. In ambystomatid salamanders (*Ambystoma opacum, maculatum and tigrinum*) bigger ova produce bigger hatchlings, which reach the feeding stage earlier and are larger during the early stages of larval growth (Kaplan 1980). In *Hyla crucifer* larger ova produce larger hatchlings (Crump 1984). In *Taricha torosa* bigger ova also produce bigger larvae which reach the feeding stage earlier (Kaplan 1985). Size differences increase with age of the larvae (Kaplan 1980). Larger *Daphnia* neonates have higher fitness rating than small offspring (Tessier and Consolatti 1989). In a more natural situation, created by allowing eggs and larvae of *Bombina orientalis* to develop in

plastic bags within natural breeding ponds, Kaplan (1989) showed that large ova produced bigger hatchlings with faster early larval growth than hatchlings from small ova. Under conditions of high predation or intense competition, these larger hatchlings and faster growing and developing larvae may have a higher survival rate than larvae that originated from smaller ova.

In the smooth newt, Bell (1977) noted that the diameter of yolked ova increased with female body size, or as he assumed, 'age'. When ova from different sized females were reared in a glass trough, eggs from larger females hatched more quickly and gave rise to larger hatchlings. Verrell and Francillon (1986) found a positive correlation between female body size and mean oocyte diameter , when they dissected ovaries from females immediately prior to reproduction. This would suggest that larger females are capable of producing larger ova than small females. However, this female size-ovum size relationship is not found in other urodeles. There is no correlation between female weight and egg weight in *Hynobius tsuensis*, and in *Cynops p. pyrrhogaster* there is actually a negative correlation, although both species show a positive correlation between female weight and clutch weight (Kuramoto 1978).

2.2 Female Body Size and Mate Choice

There are four studies of mate choice by male urodeles: Verrell (1982a), Malacarne (1984), Verrell (1986a) and Verrell (1989). All four studies show that, in a laboratory situation, males prefer larger females. *Notopthalmus viridescens* directs more courtship to the larger of two females (Verrell, 1982a); *Triturus cristatus* will preferentially court the larger of two females and also, the time spent in courtship with any individual female is correlated with female body size (Malacarne,1984); male *T. vulgaris* stay nearer to big rather than small females (Verrell, 1986a) and in a choice situation, male *Desmognathus ochrophaeus* tend to inseminate the larger of two females (Verrell, 1989).

For males there may well be an adaptive advantage in insemination of larger females, because larger females are more fecund and it has been demonstrated that if a male inseminates a bigger female then more ova are deposited (Verrell, 1986a). Theoretically,

being preferred by males should be adaptive for females. However, in the natural situation this size advantage may not be expressed. Male mate choice observed in a laboratory situation may not be representative of mating behaviour in a pond. Field observations, or observations made in semi-natural conditions are needed to establish how accurately such laboratory staged encounters reflect the natural mating situation. There may well be important differences. For example, Massey (1988) found that one of the two courtship strategies that was observed in *Notophthalmus viridescens* in the laboratory (Verrell 1982b) was not observed in nearly one thousand hours of observations made in the field. Of particular relevance to the present discussion of male mate choice, Hedlund (1990a) noted that in the field most courtship encounters in *Triturus cristatus* were terminated by the female leaving the male, and among pairs in enclosures (Hedlund 1990b) there was no evidence of male preference for larger females. It is quite possible that in the field, male newts are simply not choosy, so that variance in female R.S. will be related to fecundity rather than male mate choice.

Conclusion: Does female body size affect reproductive success? Female body size does generally seem to be related to fecundity among urodeles, since clutch size is a function of body size. However in *Triturus vulgaris* the measures of clutch size are indirect, relying on dissection of gravid females prior to oviposition, except for Verrell's (1986a) in a study that yielded very low egg numbers. The results of body size-fecundity investigations within *Triturus* species are not unanimous. There is no field evidence that suggests that female body size is a criterion for male mate choice.

2.3 Male Body Size and Reproductive Success

In this review, the effect of male body size on reproductive success in urodeles, via fighting and differential mating performance, will be considered, although it should be noted that the two may be closely linked processes.

Male body size is frequently found to be related to male reproductive success and this size effect is often mediated through male-male combat for access to females (Arak 1983 Trivers 1972, Partridge and Halliday 1984, Crespi 1989). In such encounters, large males may be at a reproductive advantage over small males. Among species where there is no combat, large male advantage may still be observed. In *Drosophila melanogaster* large males deliver more courtship to females, probably because bigger males can run faster than small males and therefore find it easier to keep up with running females (Partridge et al. 1987).

However, large male size is not always an advantage, for example when mate choice is involved. Holmberg et al. (1989) showed that female mallards chose males on the basis of the amount of display of individual males during communal display. Male weight was not related to the length of time spent in display, and females actually preferred smaller males (size measured by sternum length). In other situations, small body size can also be advantageous. It may bestow an aerobatic advantage on male flies (*Diptera* species) that facilitates pairing with females (McLachlan and Allen 1987).

Fighting in urodeles Among aggressive species, there is evidence that male body size does seem to influence fight outcome, so that larger males tend to win. In *Notophthalmus viridescens* larger males are able to displace smaller rivals during wrestling bouts (Verrell 1986b). However, the female usually escapes and, in the natural situation, Massey (1988) during almost 1000 hours of observation, did not observe this type of male-male wrestling. Initial observations of *Taricha granulosa* show that large male body size is linked to access to females via amplexus, and this may also be mediated through male-male combat (Janzen and Brodie 1989). In a laboratory study using trios of *Desmognathus ochrophaeus* (two

males and one female), Houck (1988) found that the bigger male chases off a smaller rival and then inseminates the female.

Fighting is rare among newts and certainly not reported for *T. vulgaris*. Among *Triturus*, *T. vittatus* is notable as the only species that exhibits fighting behaviour (Thorn 1968, Raxworthy 1989) but there is no quantitative information on fighting behaviour in this species. The congenerics *T. carnifex* (Cedrini and Fasolo 1971), *T. cristatus* and *T. marmoratus* (Zuiderwijk and Sparreboom 1986) have been reported as defending temporary territories, presumably to aid successful spermatophore transfer but aggression is confined to displays rather than fighting behaviour .

Body size and mating success in urodeles in the absence of fighting This subsection deals with 'mating success' rather than attempting to divide mating success into inter- and intrasexual selection, as previously defined (eg. Partridge and Halliday 1984). This is because it can be impossible to separate the confounding effects of male body size and/or male secondary sexual character size, from male and/or female motivation or performance. These confounding effects can be explained by reference to two studies that have considered the effect of male body size on courtship success in two different species in the *Triturus cristatus* group. Malacarne and Cortassa (1983) demonstrated that female *T. carnifex* picked up more spermatophores from males that had a larger tail height (the measure they used of male epigamic features). However, there was no correlation between male size (weight and the number of spermatophores picked up). This study suggests that male tail height is associated with higher mating success rather than body size. However, it is not possible to deduce whether the males with high tails were more attractive to females, which then responded positively to and induced many spermatophore depositions (mate choice) or whether the males with high tails were in some way better able to produce many spermatophores (differential male performance).

Hedlund (1990) left pairs of *T. cristatus* in enclosures overnight and recorded the number of spermatophore bases with sperm caps removed as a measure of male

reproductive success. She found that male body size was related to the numbers of spermatophores deposited early in the breeding season, but this effect was not detectable later on. Again, it is not possible to specify whether this effect is due to female preference for large males, or whether large males are in some way better able to inseminate females.

Houck et al's (1985) study of the plethodontid salamander, *Desmognathus ochrophaeus*, could find no male body size effect in laboratory trials, when males were not in combat situations for access to females. Male body size had no effect on mating success, as measured by the number of inseminations.

Body size and intrasexual selection in Triturus vulgaris. The evidence of a body size advantage within males of this species is weak and is summarized here:

Larger males have a larger testis mass (Verrell and Francillon 1986) and it is speculated that they can produce more spermatophores (Halliday 1977) and/or more sperm over the course of their lifetime than can smaller males (Halliday 1987). Halliday (1976), using principal component analysis, has shown that libido (willingness to deposit a spermatophore) is higher for males with more spermatophores - and bigger males are likely to attain high libido scores more often than small males, for the reasons outlined above. 'High libido' males were more successful in stimulating females (Teyssedre and Halliday 1986). There are no data linking body size to reproductive success in the natural situation. For example, there is no evidence that females increase spermatophore pick-up rate per encounter for larger males, but one might assume that larger males are potentially able to inseminate more females or be more successful in terms of sperm competition.

Evidence of body size effects is apparent from data drawn from different subspecies. Smaller subspecies, observed in aquaria, produce fewer spermatophores per encounter than the larger subspecies (Raxworthy, pers. comm.). *Triturus vulgaris schmidtlerorum*, the smallest subspecies produced only one spermatophore per encounter (n of 8) (Raxworthy 1988).

Another factor affecting male smooth newts during courtship, with potential consequent effects on R.S., is the need to swim to the surface in order to breathe.

Surfacing during courtship may be disadvantageous because female receptivity may decline and because a male may lose a female. So it would seem to be advantageous for a male to court without surfacing. It is known that breathing rate increases with activity levels (Halliday and Worsnop 1977) and Halliday (1977) suggests that females are exerting selection pressure on male breathing by their slow response to courtship and by tending to pick up more spermatophores deposited later in a sequence rather than early ones. Like Ultsch's (1976) sirenids, smaller males would be expected to have a higher rate of cutaneous respiration per gram body weight than bigger animals. Surface areas increase to a 2/3 power of volumetric increase (Schmidt-Nielson 1984) so that large individuals have a smaller skin surface area for gas exchange, per unit body weight than small individuals. Hence it seems reasonable to hypothesize that smaller males will be better able to prolong the time until a breathing ascent.

There are no reports of the effect of body size on male breathing rate, but of an anecdotal nature, in support of the hypothesis, is the following observation. Harris (1980) noted that the *Ambystoma maculatum* that were killed during a pond freeze, presumably due to lack of oxygen, were larger than the survivors, suggesting that the smaller salamanders were better able to withstand the low levels of dissolved oxygen. However, evidence against this trend has emerged from a study of the oviposition behaviour of *T. marmoratus pygmaeus* (Diaz-Paniagua 1989) in which inter-breath intervals tended to be longer for larger females.

Drawing again on a subspecies comparison, *T. vulgaris schmidtlerorum*, the smallest subspecies, has a lower breathing ascent rate than the nominate subspecies (Raxworthy 1988).

Courtship pheromones (*sensu* Houck 1986) Courtship pheromones have been shown to increase receptivity in female *Desmognathus ocrophaeus* (Houck and Reagan 1990). In a *Triturus* species (*T. carnifex*) courtship pheromones have been found to be produced by the abdominal (dorsal cloacal) gland (Cedrini and Fasolo 1971). In *T. vulgaris* a weak,

but not significant, correlation between SVL and the size of the dorsal cloacal gland has been found (Verrell and Francillon, 1986). Hence it is possible that larger males smell more attractive to females.

Conclusion: Does male body size affect reproductive success? There is a variety of evidence linking male body size to R.S. among urodeles. However , for *Triturus vulgaris* the evidence is indirect or speculative and studies of congenerics indicate that body size either has no effect on reproductive success (Malacarne and Cortassa 1983), or only affects reproductive success early in the courtship season (Hedlund 1990). The scarcity of evidence is probably due to the problems of observing newt courtship in the field, since newts are crepuscular or nocturnal and inhabit ponds where visibility is frequently poor. Since direct evidence is lacking it was decided that a simple test of the hypothesis that larger males can inseminate more females was simply to record the maximum number of spermatophores that males of different body sizes were able to deposit during one laboratory controlled encounter. The working hypothesis is that larger males can deposit more spermatophores than smaller males. This study is reported in section 3.2.

Chapter 3

Investigations of the Relationship between Body Size and Individual Reproductive Success

3.1 Female Body Size and Fecundity

Introduction In Chapter 2 it was concluded that measures linking fecundity and female body size were either indirect (ovarian oocyte counts) and not unanimous, or yielded low numbers of ova (Verrell 1986). From the the general trend within other urodele species it would seem that female body size is very likely to be correlated with clutch mass. However, the data on actual numbers of ova oviposited by individual females would provide more information on the nature of this relationship, resolve any ambiguities and reveal whether females do actually oviposit all the oocytes that are yolked at the beginning of a breeding season.

Costs associated with ovipositional behaviour of *Triturus* species have not been investigated, but observation of smooth newts in a natural pond in the Milton Keynes area suggests that such costs may exist. Towards the end of one reproductive period (May), females were seen ovipositing during daylight hours. Female smooth newts use their hind feet to wrap single ova in plant leaves or other flexible matter (Smith 1973), so that females in this particular pond were easily visible in shallow water, at the pond's edge searching for oviposition sites and were also seen ovipositing just below, or at, the water's surface. This latter operation sometimes involved individuals rolling onto their sides and backs, clearly exposing the non-cryptically coloured ventral surfaces. Such behaviour may expose females to greater risk of predation than does their otherwise secretive nature. In an observational study of oviposition behaviour in *Triturus marmoratus pygmaeus*, Diaz-Paniagua (1989) found that large females were more efficient egg-layers, in terms of ova produced per oviposition attempt and that they also invested less time in failed attempts than smaller females. She sees the potential advantages to large female *Triturus marmoratus*

pygmaeus as being energetic savings and leaving more time in which to search for new oviposition sites. However, if there are increased chances of predation associated with ovipositional behaviour, then efficient oviposition may also be advantageous in minimizing these dangers. Diaz-Paniagua's work predicts that the most efficient ovipositors will be large females.

The following study aimed to test three hypotheses:

1. Female body size is positively correlated with the size of individual ova oviposited.
2. Female body size is positively correlated with individual clutch size (number of ova oviposited).
3. Female body size is positively correlated with efficiency of oviposition (rate of ova deposition).

Female *Triturus* produce individual eggs, sequentially over a period of days, making precise counts difficult. Also the number of spermatophores that a female has accepted may affect the number of ova produced (Verrell 1986a). The present study examined ovum sizes for females of varying body size within one population over two years (1988 and 1989) and clutch size and temporal pattern of oviposition activity during one of those years (1989).

Methods In 1988, 53 female newts were collected in the Milton Keynes area from two ponds (Great Linford and the Open University [O.U.] campus). The females were split into three groups and each group was maintained in a glass holding aquarium (30 x 45 x 25 cm) with a thin layer of gravel covering the bottom, and bricks and broken clay pots under which the newts could hide. *Tubifex* and zooplankton were provided as food. The aquaria were maintained in the newt room of the Open University. This was kept at a constant temperature of 12°C with a photoperiod of 16:8 L:D and a 'dusk' period of one hour of subdued light immediately prior to lights on and immediately after lights off. To record which ova were oviposited by individual females, newts have to be maintained individually. Single newts were transferred to similar aquaria to those described above.

Artificial 'weed' was provided to allow females to oviposit and at the same time facilitate ease of collection. This 'weed' consisted of strips of polythene (20 x 2 cm) which were cut along one edge to create a fringe. Weights were placed on one end of each strip to anchor the 'weed' to the substrate. Weed was inspected daily for ova. However, most animals rapidly lost condition and no ova were produced. After five days of no ova production, these females were released and replaced with others from the holding aquaria. Males, captured in funnel traps at the OU pond, were introduced every week, for two or three days, to ensure that all oocytes were fertilized. After the two or three days these males were returned to the pond.

Ova were collected every 1-3 days. Ova were removed from the substrate, by gently peeling away the polythene, and fixed in 4% buffered formalin. The jelly coat was then removed from each ovum with a scalpel and forceps and the ovum measured under a stereo microscope with a graticule eyepiece. The mean of the widest and narrowest diameters was calculated to provide a measure of ovum diameter. Other studies have established that ovum diameter is significantly correlated with dry mass (Crump 1984 and Crump and Kaplan 1979). Only uncleaved ova were measured, since some studies have shown that ovum size can increase with ontogeny (Kaplan 1979). First cleavage occurs in a matter of hours (pers. obs.), and since most oviposition occurred during the night, it was difficult to obtain large numbers of uncleaved ova from any single female.

Once a female had finished oviposition (again using the criterion of five days with no ova produced) she was anaesthetized and SVL was measured to the nearest 0.5 mm. It was considered necessary to anaesthetize newts in order to obtain accurate linear body measurements. Anaesthesia was achieved by immersion in a 1:1000 solution of MS-222 (Sandoz), until newts became unresponsive to touch by forceps. They were then removed from the anaesthetic solution, rinsed in tap water and measured by stretching out to full length on a mm ruler. The newts were then placed in running tap water, which seems to increase speed of recovery from anaesthsia. SVL of the females in the present study was measured after oviposition to avoid the possibility that anaesthesia may have some effect on

adaptation to aquatic life or normal ovipositional behaviour.

In 1989 facilities were made available to maintain newts outdoors. 12 females, selected to cover a wide range of body sizes, were collected at a drift fence as they moved towards the O.U. pond (7-3-89 - 27-3-89). These were individually maintained outside in plastic aquaria, measuring (39 x 20 x 25 cm) placed in a larger tub of water (see diagram in Fig. 3.1). These tanks were similarly furnished to the tanks used in 1988, with one clay pot refuge and some artificial weed in each. Females were judged to have completed oviposition on the basis of external characters and behaviour rather than date of last oviposition. The criteria used to judge completion of oviposition were:

1. Reduction of the tail fin.
2. Skin becomes granular rather than smooth.
3. Cloaca becomes reduced in size and dome-shaped rather than flat-topped dome-shaped.
4. Floating at water surface.

Note that these characteristics are also associated with resumption of terrestrial life. The following female measurements were taken:

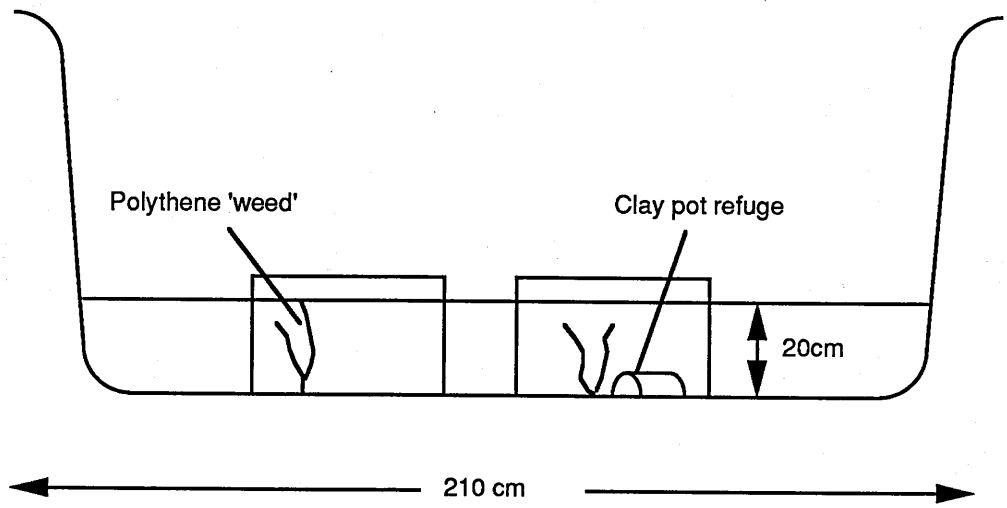
Initial wt. = weight on day of first oviposition (g).

Final wt. = weight at end of oviposition period (g).

SVL = snout vent length (length from nose tip to hind most point of cloaca [mm] taken from females anaesethetized in MS-222, after completion of oviposition).

Ovum sizes were measured as in 1988, attempting to obtain a sample of 10 ova from each female.

Aquaria housing individual female smooth newts (see Section 3.1)



Aquarium housing male smooth newts (see section 3.2)

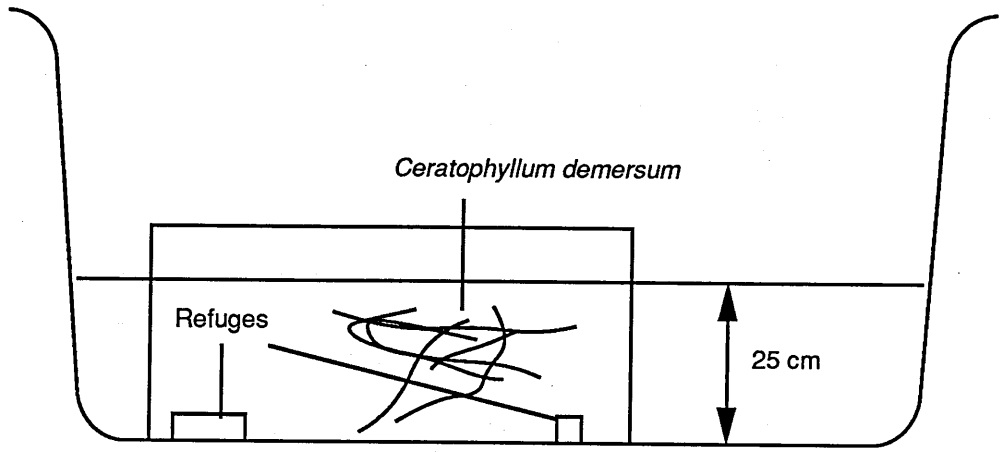


Fig. 3.1. Diagram to show newt tanks within large tubs

Results In 1988 11 out of 53 females deposited ova. Of these, two females deposited only 2 ova, so these data were excluded from analysis as ovum sample sizes were so low as to potentially obscure any body size-ovum size relationship. The remainder of the sample sizes varied between 4 and 22 ova per female. In 1989 10 out of 12 females produced large numbers of ova (88-637). Samples of uncleaved ova measured varied from 6 to 16 per female. Descriptive data of the sizes of the females that oviposited in 1989 are given in Table 3.1. below.

	Mean	s.d.
TL (mm)	85.5	8.22
SVL (mm)	45.9	3.816
Initial wt. (g)	2.68	0.822
No. ova collected	300	189.4

Table 3.1 Mean body size and mean number of ova produced by ten *T. vulgaris* that oviposited in 1989.

To test the first hypothesis, that female body size is positively correlated with the size of individual ova oviposited, regressions of mean ovum diameter on female SVL were performed on the data sets obtained over the two years, using data from 9 and 10 newts respectively. Regression analyses of mean ovum diameter on female SVL:

	p value of regression	r	p value of r
1988	0.513	+0.25	> 0.05
1989	0.023*	+0.704	< 0.05*

Regression analysis of mean ovum diameter on initial wt. of female:

1989	0.448	+0.49	> 0.05

In 1988 the results show a slight positive, but insignificant relationship, whereas in 1989 there is a significantly positive relationship between female SVL and mean ovum diameter and a positive but insignificant relationship between female initial wt. and mean ovum diameter (see Fig 3.2).

The remainder of the data analysis concerns oviposition in in 1989 only, since it was not possible to collect comparable data in 1988. To test the hypothesis that female body size is positively correlated with individual clutch size (number of ova oviposited) a regression analysis was performed on the number of ova produced on female body size (SVL and initial weight), using data from all 12 newts.

	p value of regression	r	p value of r
No. ova on SVL.	0.020**	+0.66	< 0.05*
No. ova on initial wt.	0.007**	+0.73	< 0.01**

To summarize: female body size is positively correlated with the number of ova oviposited. Across the size range of the population clutch size increases (see Figs. 3.4 [a] and [b]). From the equation of the regression line in Fig. 3.4.(a) ($y = -1265.8 + 33.6x$, where x = the SVL of the female and y = the number of ova oviposited) it is apparent that over the size range of the ovipositing females (40-52mm SVL), clutch size increases six fold.

Since there was a slight tendency for ovum size and female body size to be positively correlated and there was a significant positive correlation between body size and the number of ova oviposited, it would suggest that a positive correlation may exist between ovum size and ovum number. A Pearson product-moment correlation coefficient was used to test this hypothesis, with the result that there is a significant, positive correlation between individual ovum size and the number of ova in a clutch ($r = +0.72$, $p < 0.05$).

It was noted that females tended to lose weight over the period of oviposition. Is this

weight loss directly related to oviposition?

Regression analysis of number of ova oviposited on change in weight for 10 ovipositing newts:

	p value of regression	r	p value of r
No. ova on change in wt.	0.053	+0.63	p = 0.05*

See Fig. 3.5(a)

Conclusion: female weight loss is related to number of ova produced.

If larger females produce more ova and weight loss is also related to number of ova, then larger females would also be expected to lose more weight over the oviposition period.

Regression analysis of weight change on initial weight for the 10 newts that produced ova:

	p value of regression	r	p value of r
Weight change on initial wt.	0.03	+0.68	p < 0.05*

Conclusion: larger females lose more weight during oviposition. See Fig. 3.5(b)

Are larger females able to invest relatively more in oviposition than smaller females?

Regression analysis of relative weight loss (% wt. change on initial wt.).

	p value of regression	r	p value of r
% wt. change on initial wt.	0.282	+0.38	p > 0.05

Conclusion: trend for larger females to invest relatively more in oviposition than smaller females, but not statistically significant. See Fig 3.5(c).

Efficiency of oviposition To test the third hypothesis that larger females can oviposit at a faster rate than smaller females, rate of oviposition was calculated for each female. Number of ova deposited per day was calculated as clutch size divided by oviposition period, where oviposition period is the number of days elapsed between the first and last ovum produced by each female. A regression of mean rate of oviposition (ova per day) was performed on female body size (initial wt.).

	p value of regression	r	p value of r
Rate of oviposition on body size	< 0.000	+0.89	p < 0.01**

Conclusion: Larger females have a faster rate of oviposition than small females. (See Fig. 3.6).

However, faster rate of oviposition does not reduce the length of time, in terms of days, invested in oviposition since a regression of length of oviposition period on initial weight produces no particular trend:

	p value of regression	r	p value of r
Length oviposition period on body size (initial wt.).	0.864	+0.063	p > 0.05

Conclusion: There is no relationship between female body size and duration of oviposition period.

* = significant at 5% level.

** = significant at 1% level.

Variation in ovum size Coefficients of variation in ovum diameter varied between 2.021 and 4.034 for each female in 1989 and did not appear to vary in any systematic way among females. The coefficient of variation for all ova measured in that sample (114 ova) was 4.364. In a between clutches comparison, the largest mean ovum diameter was only 9.6% larger than the smallest mean ovum diameter.

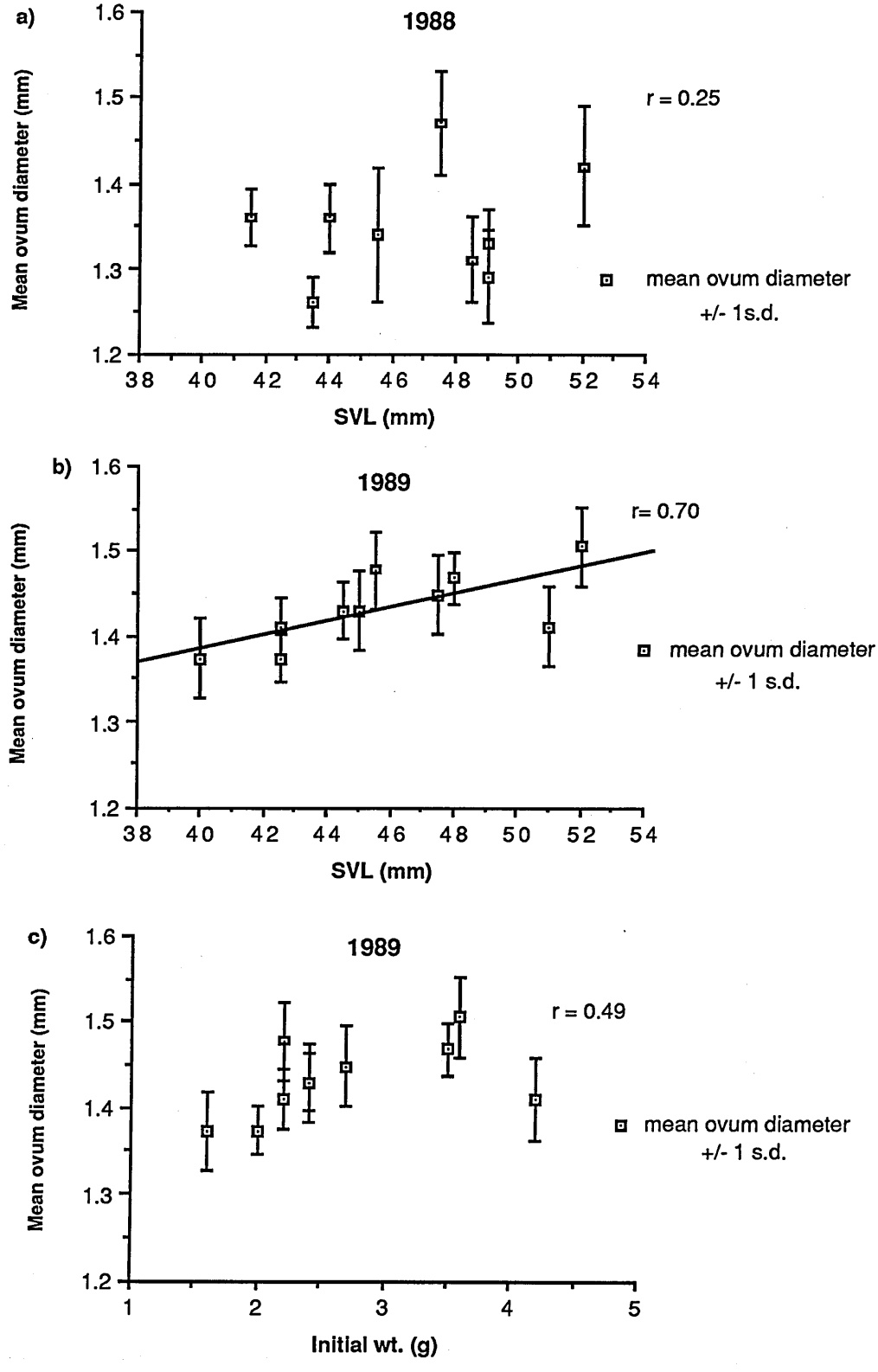


Fig. 3.2. Graphs to show relationship between female body size and ovum size in the smooth newt. a) Ovum diameter plotted against SVL (1988). b) Ovum diameter plotted against SVL (1989). c) Ovum diameter plotted against intial weight of female (1989).

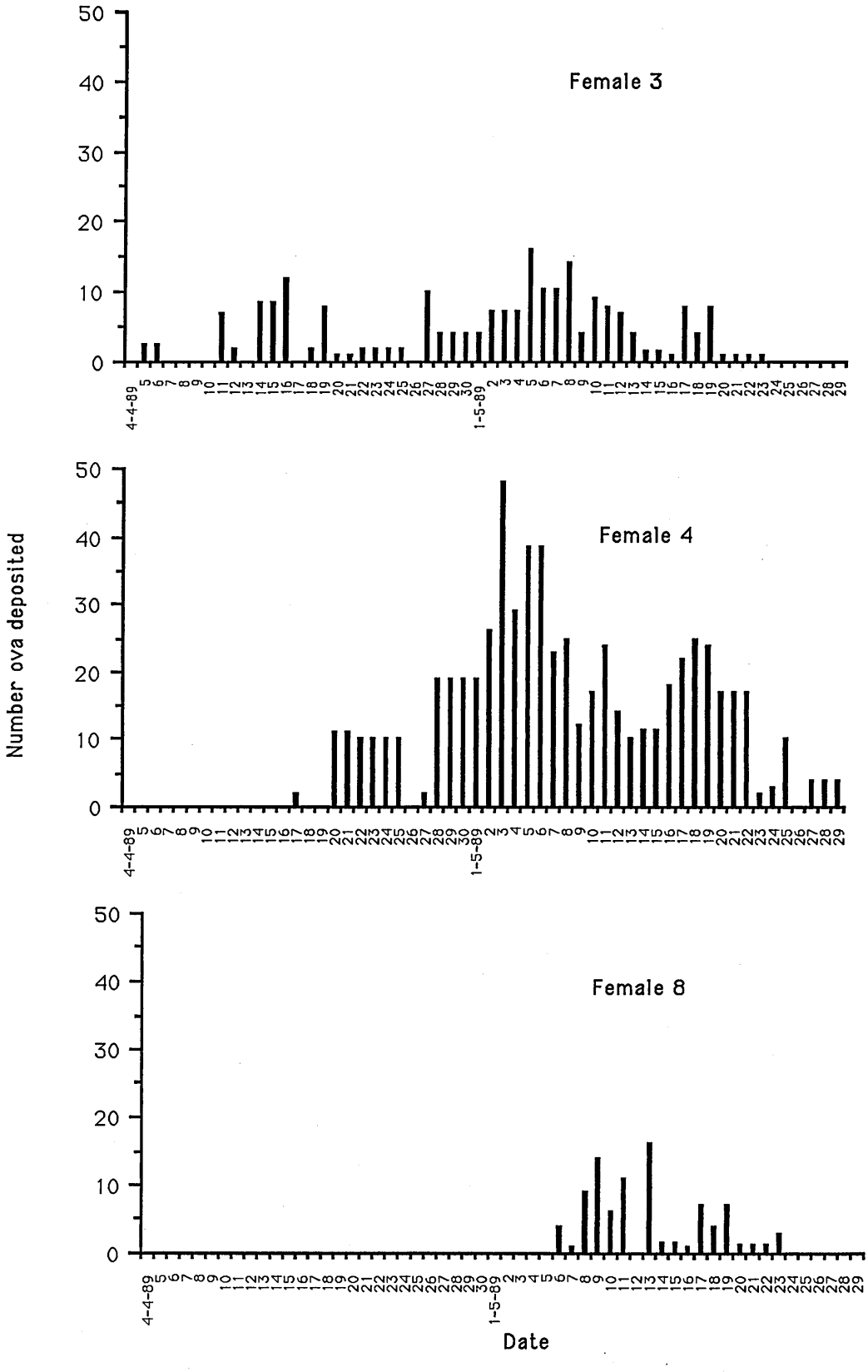


Fig. 3.3 To show oviposition time course of three smooth newts. Data from these three particular females are shown to illustrate individual variation in clutch size and timing of oviposition.

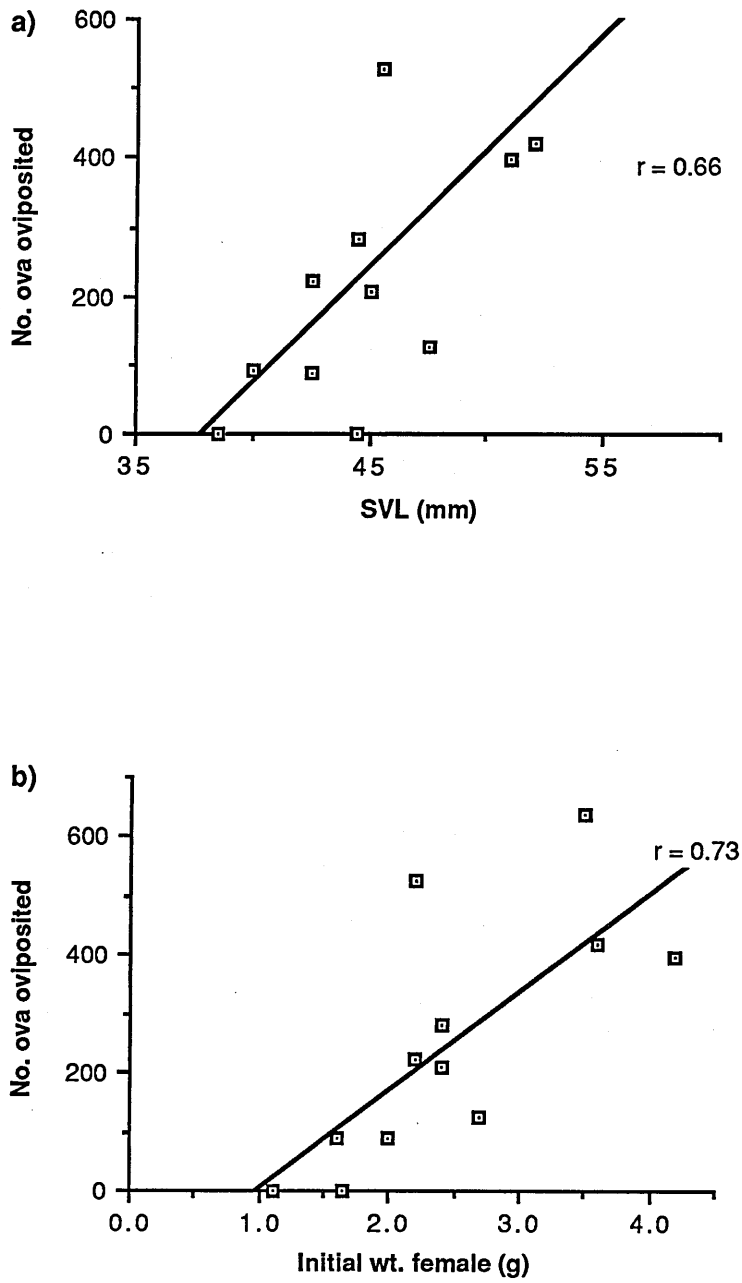


Fig 3.4. Graphs to show relationship between female body size and clutch size in *Triturus vulgaris*.

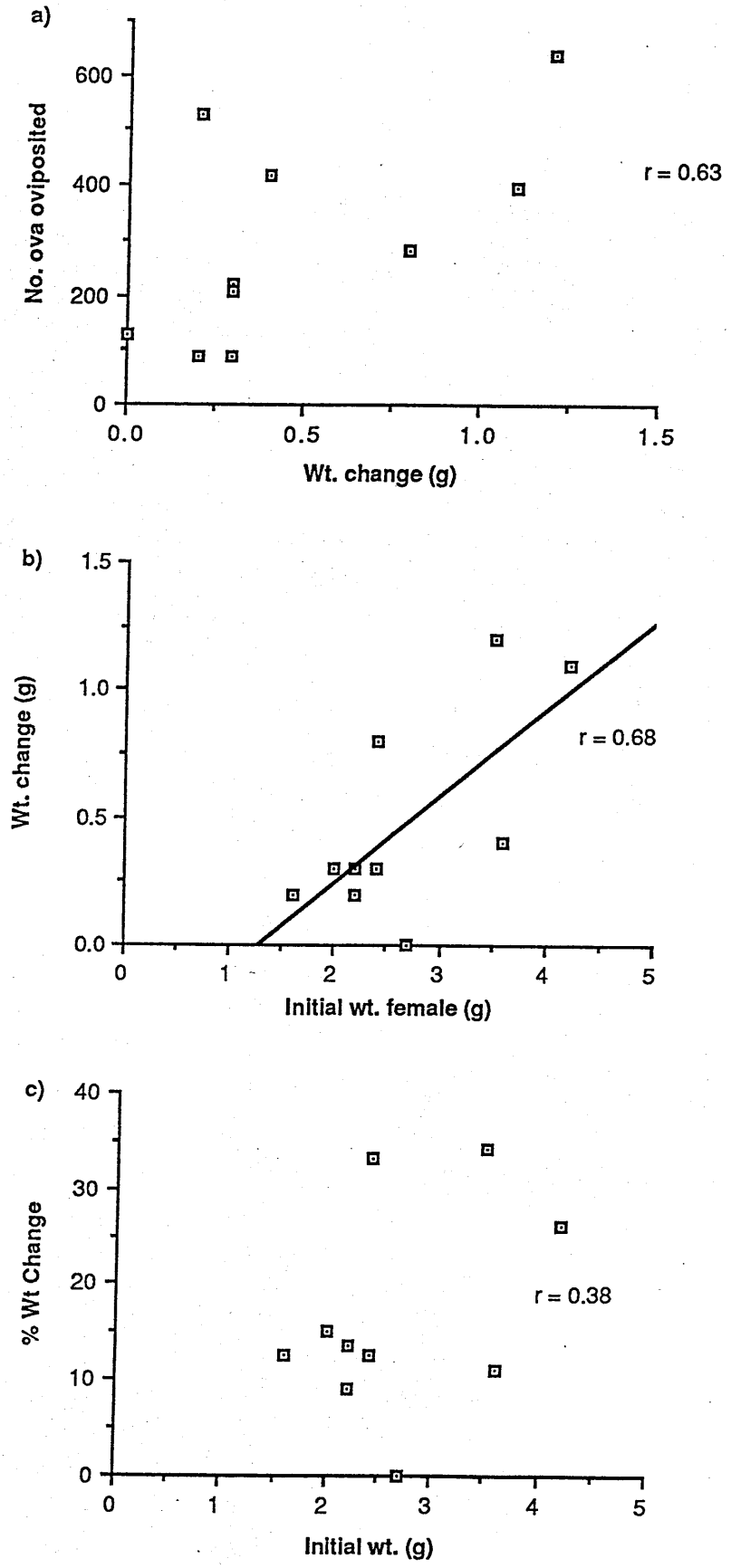


Fig 3.5. Graphs to show (a) relationship between female weight loss over oviposition period and clutch size. b) Relationship between female body size and weight loss over oviposition period. c) Relationship between female body size and relative weight loss over oviposition period.

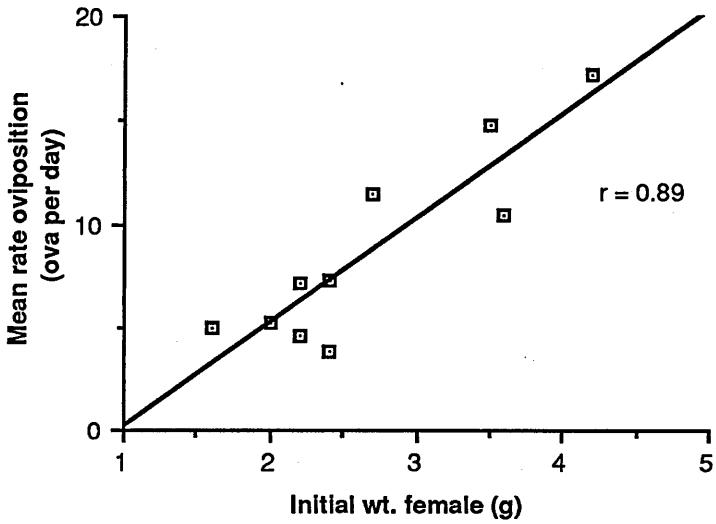


Fig. 3.6. Graph to show mean oviposition rate plotted against female body size (initial weight).

Discussion In 1988 only 11 out of 53 females deposited ova. The difficulty in persuading female newts to oviposit under captive conditions has been indirectly noted elsewhere. Wimpenny (1951) performed a series of experiments, providing *Triturus vulgaris* and *Triturus cristatus* with different oviposition substrates and concluded that oxygen production by water plants induced spawning and helped to maintain newts in the aquatic phase. However, his studies seem to be so strongly affected by the loss of reproductive condition of his newts that his conclusion does not seem to be correct. Firstly, Wimpenny did actually find some ova produced by animals in the absence of weed (which he attributes to the growth of green algae). Secondly, smooth newts will breed in totally weedless ponds (a disused swimming pool, pers. obs. and Hagström 1979) as will crested newts (Smith 1973), or in stagnant ponds where oxygen levels are presumably very low (pers. obs.). In addition, the newts from the present 1989 study oviposited large numbers of ova in the absence of pond weed. Thirdly, Wimpenny notes that all of his newts tended to lose condition after about four days, and his observed clutch sizes were very low (22 ova from 8 females and 43 ova from 56 females). These results are similar to those obtained in 1988, during the present study, but the assessment of the responses of the newts is different. It is concluded here that wild-caught newts rapidly tend to lose condition in the aquarium situation as described here, and also as described by Wimpenny (1951). Rapid loss of condition of female newts maintained in captivity may also explain why Verrell (1986a) recorded such low clutch sizes (25-80 ova) for smooth newts after one insemination. An experiment comparing the oviposition of females inseminated once, with controls that have been inseminated multiply, is needed to establish whether smooth newts require multiple insemination in order to fertilize a whole clutch. Loss of condition may also explain why Arntzen and Hedlund (1990) recorded such low rates of oviposition. They recorded rates of 0.33 eggs per day for *T. cristatus*, 0.74 for *T. marmoratus* and 1.11 for hybrids. From their estimate of clutch size of 189 ova, it would take a female *T. cristatus* 573 days to oviposit a whole clutch. Maintaining newts outdoors, and hence in semi-natural conditions, seems to allow newts to exhibit a more natural representation of

their true behaviour. Conclusions drawn from studies of newts that have been removed from their natural habitat should be treated with caution.

The clutch sizes recorded in 1989 (88-637 ova) are of a similar order of magnitude to estimates of clutch size generated from ovarian oocyte counts, from populations in Oxfordshire or Buckinghamshire, made at the beginning of a breeding season (approximately 100-400 recorded by Bell [1977], 100-500 recorded by Verrell [1986a], 130-470 from graph in Verrell and Francillon [1986]). Thus I would conclude that female *T. vulgaris* from local populations are quite able to oviposit all ova yolked at the beginning of a season, during that season. Harrison (1985) working on *T. vulgaris* at Llysdinam, mid-Wales, recorded mean clutch sizes of 239 (yolked ova carried by immigrating females) or 190 (obtained by subtracting yolked ova in emigrating females from number in immigrating females) which seem much lower than the mean in the present study (300). However the Llysdinam newts are smaller than those found in the Milton Keynes area, with mean total length (TL) of 79 mm for immigrants and 76 mm for emigrants. The difference in clutch sizes between local newts and those from mid-Wales may be a reflection of differences in body size.

Hagström's (1980) lower ovarian counts of approximately 200 may reflect the shorter foraging and/or breeding seasons of *T. vulgaris* in Sweden. Nevertheless, this does not explain why females did not appear to oviposit all yolked ova or why Harrison captured emigrating females that still contained yolked ova.

Harris (1987) notes that female *Notophthalmus viridescens dorsalis* actually oviposit rather more ova than ovarian oocyte counts would predict. Harris interprets this observation as evidence that females may be able to yolk up ova during the oviposition period, depending on food availability. His interpretation of this finding is equally applicable to the present study and does not seem an unreasonable conclusion when it is considered that adult smooth newts are reported to perform most feeding and annual growth, as well as courtship, during the aquatic phase (Verrell 1987). Such a pattern of vitellogenesis would also explain the presence of yolked ova in the ovaries immediately

after the breeding period (Hagström 1980, Harrison 1985 and Verrell et al. 1986)

Variance in Ovum Size Among urodeles, larger eggs are suggested to have a greater fitness, which was the assumption made during this investigation (see Chapter 2). However if larger ova produce faster growing larvae then why does this observed variation in ovum size exist in this population? There are a multitude of factors that may determine this variance, and there is much discussion as to its causation. Within a species, there are two alternative models of propagule size: optimality and plasticity. The optimality approach incorporates some of Lack's observations (1954) and these have been presented more formally and graphically by Smith and Fretwell (1974). The main points of the latter's model are the assumption that there is an optimum amount of resources that a particular female should allocate to reproduction in a particular situation, hence energy available for reproduction is finite. Note that in birds, the finite limit to 'energy available for reproduction' is not set by resources available to a female at egg-laying, since birds can produce more eggs to compensate for those removed from a clutch, but by parental ability to feed the nestlings (Lack 1954). However, actual clutch mass incubated is finite. It then follows that there must be a trade-off between propagule size and numbers. Optimum propagule size will be dictated by the demands of the environment. In order to produce optimum sized propagules, females will alter clutch size accordingly. Evidence for this sort of effect is provided by birds, which tend to vary clutch size, but not egg size (Lack 1954). The plasticity approach, (Capinera 1979, Kaplan and Cooper 1984) concludes that parental fitness will be maximized via variable propagule size. This latter approach is particularly relevant to unpredictable environments, where optimum propagule size may also be unpredictable, with the consequence that a 'bet-hedging' strategy - that is to say production of propagules of varying size - will yield higher parental fitness (Kaplan and Cooper 1984). Certainly, situations can be envisaged where fast-developing larvae would be favoured - ephemeral pools for example. But it is also easy to imagine situations where this 'big ova produces fast developing larva' effect would be of no advantage, for example

in permanent ponds, or conditions of low competition or predation. In such ponds, fast developing larvae would not be able to exploit the rapid growth environment to the full. There is some evidence to support the hypothesis that different sized ova have evolved as a response to different growth environments. In the field, Woodward (1982) found that *Ambystoma maculatum* breeding in permanent pools produced more, but smaller, ova than individuals in temporary ponds. In the laboratory, Berven and Chadra (1988) rearing *Rana sylvatica* found big ova always produced larvae which reached metamorphosis faster. At low densities and high food levels, however, the larvae from the smaller ova actually attained a greater size at metamorphosis than the large, faster developing larvae. Kaplan (1985) recorded differential growth responses by larvae from different egg sizes under different growth conditions in *Taricha torosa.* Big eggs produced big larvae which attained metamorphosis before smaller larvae from smaller eggs - but only in an unlimited food situation. When food was provided at a lower rate, the bigger larvae took longer to develop. So, it is feasible that egg size may be subject to a mixed strategy selection pressure. Different size eggs may fare differentially as environmental conditions change.

Environmental unpredictability may thus select for variance in ovum size rather than a single optimum. *T.vulgaris* faces two types of unpredictability. Firstly, it breeds in small and often temporary ponds. This type of habitat varies temporally due to climatic conditions, which are stochastic rather than predictable, and spatially, since two closely neighbouring ponds can produce different larval growth responses (eg. Savage 1952, Newman 1989). Secondly, the complex life cycle of newts means that vitellogenesis and oviposition occur in different environments. *T. vulgaris* spends most of its period of vitellogenesis in a terrestrial habitat (Verrell et al. 1986), whilst oviposition and larval growth occur in a lentic environment. Hence a female has no opportunity to assess the nature of the larval environment and adjust ovum size accordingly. Hence there are good *a priori* reasons to assume that bet-hedging might be selected as a response to an inability to predict the nature of the larval growth environment.

When examining variance in propagule size, it must be decided whether variance within a clutch is adaptive (ie. bet-hedging) or a reflection of variation typical of most

biological traits and a reflection of developmental constraints. In the present study the coefficients of variation were fairly low (2.021-4.034) within each clutch and still fairly low (4.364) for the whole population. Between clutches, mean ovum size only increased by 9.6% between the smallest and largest means. Tessier and Consollati (1989) concluded that coefficients of variation observed in the body lengths of *Daphnia* clone neonates, which were between about 3 and 7%, were small enough to be attributed to variance typical of biological constraints. Hence there may be no need to invoke selection for genotypic variability to explain the phenotypic variability observed. If this is the case, then the variance in ovum size recorded in *T. vulgaris* may just be 'noise' around an optimum value rather than adaptive variance.

Production of ova of an optimal size seems to be at odds with the observation that larger individual females tend to produce, on average, larger ova as found in 1989. This positive relationship was also predicted by Verrell and Francillon's (1986) data on ovarian oocyte sizes. The same relationship has been recorded in some fish species, but with no explanation of why it should occur (Wootton 1990). One possible explanation is that ovum size is subject to morphological or developmental constraints. Egg size may be limited by size of the female oviduct or cloaca, which would produce a positive correlation between female body size and mean ovum size. This seems to be unlikely to be acting on the ovum size of *T. vulgaris*, since within the morphologically conservative urodele taxa, some species can produce few, but very large eggs, for example *Desmognathus*.

McGinley (1989) postulates a model to explain the same phenomenon, but this depends on the assumption that mean probability of survival increases with clutch size (eg. sea turtles swamping predators). Again this does not seem to be relevant to smooth newts which are not likely to be able to swamp predators in the same way because of their asynchronous oviposition.

As an alternative, perhaps optimum ovum size varies with the quantity of resources that each female allocates to reproduction. If offspring viability increases with parental investment, then there may be limiting returns on this investment, as suggested by Smith

and Fretwell (1974) and as demonstrated by Tessier and Consolatti (1989) for *Daphnia*. Adopting an approach employed in models of optimal foraging (see Krebs and McCleery [1984]), the relationship between propagule size and propagule fitness can be represented by the curve shown in Fig. 3.7(a). This trade-off curve is based on the fact that offspring fitness declines as egg size decreases (see Ch. 2.). The shape of the curve is similar to that found by Tessier and Consolatti (1989) for the increase in fitness of *Daphnia* clones as neonate size increases.

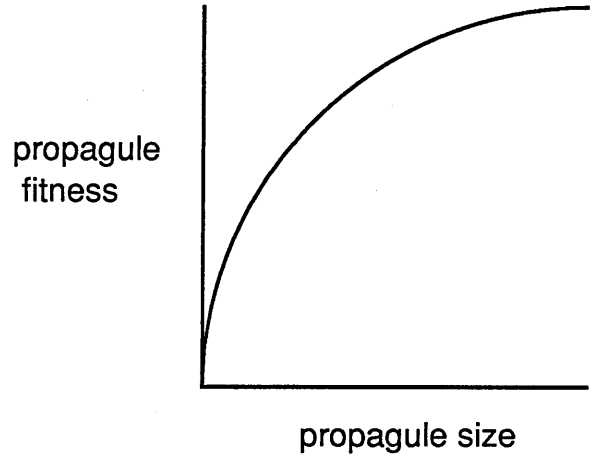


Fig. 3.7. Curve to show hypothetical increasing fitness of propagule with increasing propagule size.

To maximize parental fitness each female should be selected to optimize offspring fitness per total investment. In Fig. 3.8, female investment in propagule formation is given the value *a*. This value, *a*, is represented on the x axis of the graph which also shows the trade-off curve for propagule fitness and size. Propagule fitness per energy invested is a value that can be calculated as the gradient of the line *ax*. The maximum value of this gradient is represented in Fig. 3.8.

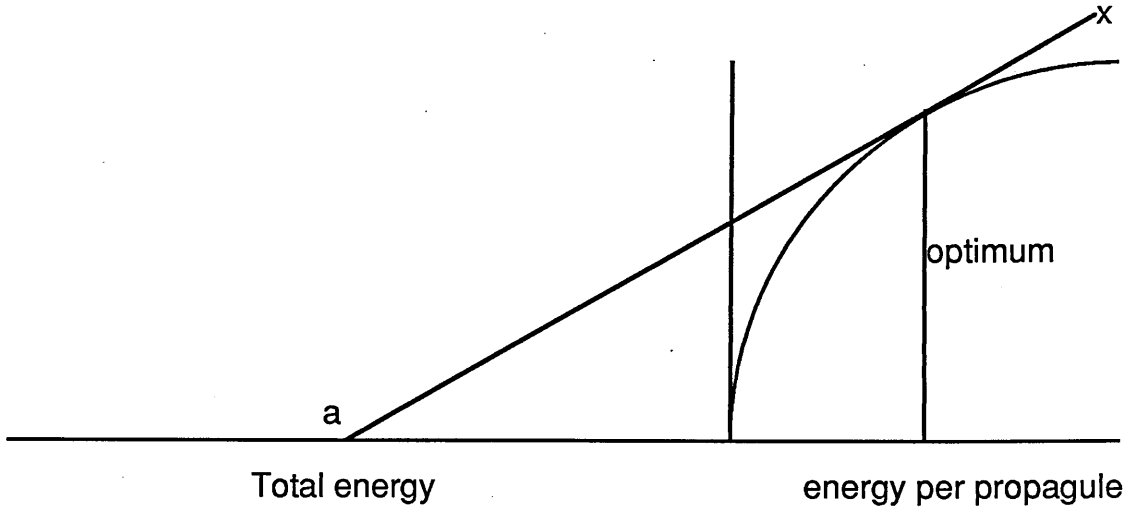


Fig. 3.8. To show optimum ovum size for a fixed amount of maternal investment (*a*).

Fig. 3.9 represents a situation in which two females have different amounts of resource to invest in propagules. The two different amounts of resource are labelled as *b* and *c*. If all offspring in a given environment are subject to similar selection pressures then all offspring will be subject to the same trade-off curve. Maximizing the gradient of the lines *by* and *cz* will give different optima for different initial investments. A female investing more in reproduction will have a larger optimum propagule size than a female with a smaller amount of resources to invest. In species in which large females invest more, in absolute terms, than do small females then it might be expected that these larger females should produce slightly larger propagules.

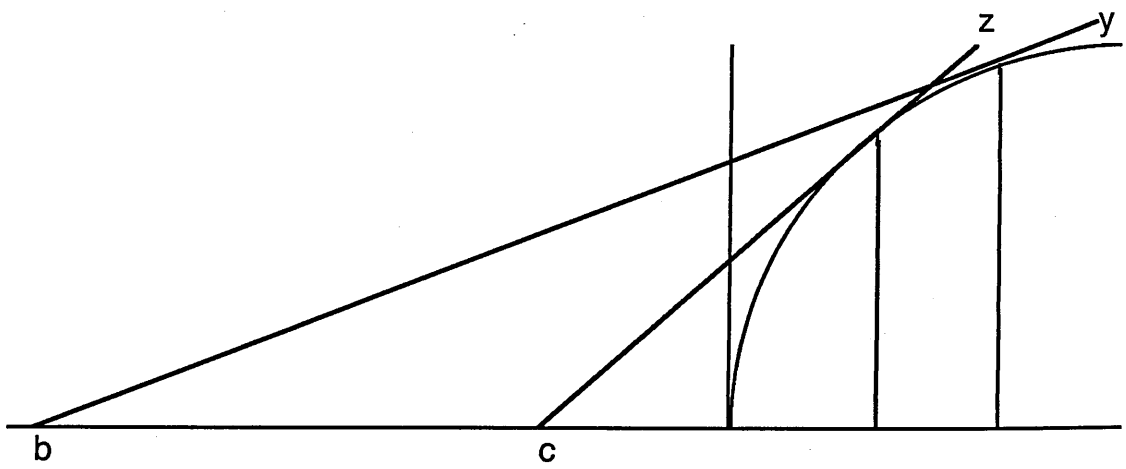


Fig. 3.9. To show optimum propagule sizes for differing amounts of resources invested in reproduction.

Oviposition Time Course There was no obvious pattern to the sequence of oviposition. Fig. 3.3 shows oviposition time courses of three individuals, demonstrating variability in oviposition pattern. There was much variance in the timing of initiation of oviposition, length of oviposition period and date of completion of oviposition. The first ovum appeared on 4-4-89 and the last on 26-6-89, a period of 84 days. Smith (1973) gives three months as the oviposition period for British newts and Diaz-Paniagua gives 2.5-3 months for *T. marmoratus* in the wild (1989). Bell and Lawton (1975) report a different scenario for *Triturus vulgaris*. They propose that oviposition occurs in three peaks. A small early peak of autumn migrants, and second and third smaller peaks from the spring arrivals. The main peak lasts for only 43 days, with the total period lasting about 125 days. However, the assertion that oviposition occurs in three waves is suspect, since the method used to calculate date of oviposition of ova involved the estimation of date of laying using degree days, which rests on the assumption that temperature remains constant over the oviposition period. In practice, water temperatures experienced by ova in ponds fluctuate on a diurnal basis and over the course of the oviposition period (pers.obs.). Also, the authors themselves note that their data may be interpreted as a normally distributed, single curve as well a trimodal curve. Results of the present study suggest that there is a large variation in the timing of individual oviposition, which when translated to the population level produces a prolonged period of oviposition. The only general pattern detectable is that for each individual, the rate of oviposition starts fairly slowly, builds to a peak and then declines before termination of the whole sequence. Much of the pattern of oviposition is probably dictated by temperature. For example, on the night of 25-4-89, temperature dropped to freezing, and this appears to have interrupted oviposition, since no ova were found on 26-4-89 (see Fig 3.3, females 3 and 4). Females also seem quite able to interrupt oviposition, for periods of up to 15 days, and then resume (see oviposition time course for female 5 in Fig 3.10). Why this should occur is unclear, but it stresses the importance of using morphological changes to determine the end of an oviposition sequence.

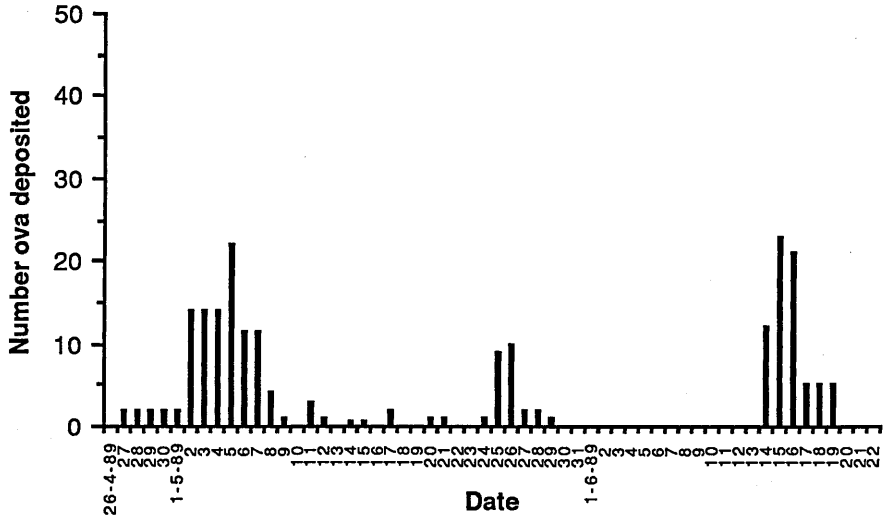


Fig. 3.10 Oviposition time course for female 5 to illustrate interruptions in oviposition sequence.

Female Body Size and Clutch Size Female body size, both SVL and wt. were strongly correlated with clutch size, with clutch size increasing six-fold over the size range of females in the study, which represents the range in a natural population. This confirms that Bell (1977) and Verrell and Francillon's (1986) size-related predictions based on ovarian oocyte counts are valid. Females lost weight over the oviposition period and the magnitude of weight loss was related to the number of ova produced, so large females, since they were producing more ova, tended to suffer greater absolute weight loss. Whether larger females were actually investing more, in terms of relative body weight, in oviposition than were the smaller females is uncertain, since the tendency to do this was positive, but not significant. However, Fig. 3.6 shows that egg number seems to increase faster than body weight, which does lend more credence to the hypothesis that larger female smooth newts can invest relatively more in reproduction than can smaller females. This would certainly fit the prediction of Reiss (1989) that for iteroparous species, with indeterminate growth, energy allocated to reproduction increases at a faster rate than body weight, as it does within fish species. The theoretical reason he provides to explain this is that smaller females invest more of their energy budget in growth than do larger (and more slowly growing)

females.

The lack of significance of the regression of percentage weight change on initial weight (see Fig. 3.5 [c]) may be a reflection of the ability of larger females not only to be able to produce more ova per gram body weight, but also to be able to recover from this energy expenditure rather more rapidly than smaller conspecifics.

The positive correlation between ovum size and number contradicts the expectation of a trade-off as predicted by Lack (1954) and Smith and Fretwell (1974). VanNoordwijk and de Jong (1986) provide a theoretical explanation of how positive correlations such as this can occur, instead of the more conventionally predicted negative correlations. They claim that if variation in quantity of resources assimilated by individuals is greater than variation in allocation strategies between two functions (ovum size and number in this case), then positive correlations are possible. This argument rests on acceptance that some individuals are in some way 'better' than others, in terms of resource accrual. There is some evidence of such positive correlations from other species. In great tits, females that produce a large clutch one year also tend to produce a large clutch the next year, irrespective of territory quality (Norris 1990). Differences in resource accrual could be used as a measure of how 'good' individuals are. Applying this theory to smooth newts, larger females produce more and bigger ova because they have more energy available for reproduction. This also fits neatly with the hypothesis proposed on p.15 that size of adult smooth newts is dictated by ability to assimilate resources during the pre-maturity stages. It seems reasonable to assume that individuals that are innately poor at resource accrual during the juvenile stage will also be so during the adult stage.

Variation in oviposition rate Large females tended to produce more ova on average per day in the oviposition period than smaller females. It is not possible to tell if this is a size-related physiological effect or whether some behavioural factor is responsible, but Diaz-Paniagua's (1989) work suggests that this effect may be behaviourally mediated. The effect of body size on oviposition rate in the present study is quite compatible with her

results, so perhaps small *Triturus vulgaris* are also behaviourally more inept at oviposition. However, although larger females were able to oviposit at a higher rate than small females, any advantage obtained in terms of reduced predation risk or energetic saving is debatable, because their larger clutch sizes meant that they are just as likely to spend as many days in this behaviour as small females. It is not possible to say whether variation in body size, and hence oviposition rate, is associated with differential use of time budgets within this period. Large females may be more efficient ovipositors and hence spend less time ovipositing each ovum, so that both large and small females spend the same total length of time in oviposition activities. Alternatively, there may be no difference in the time spent per ovum, between large and small females, so that large females devote more of their total time budget to oviposition.

Summary To conclude, female body size appears to be strongly related to fitness via differential fecundity. Fecundity increases at a faster rate than does body size. Large females produce more, and slightly larger, ova than do small females. Large females also have higher rates of oviposition.

3.2 Body Size and Short-Term Limitation to Male Mating Capacity in the Smooth Newt (1988 Study).

Introduction Chapter 2 summarized arguments that linked male body size to R.S. and concluded that there was no direct evidence for such an effect in *Triturus vulgaris vulgaris*. The following studies, from two years (1988 [Section 3.2] and 1989 [Section 3.3]) were designed to examine the effect of body size on mating capacity of male smooth newts.

Male reproductive success is maximized by successful insemination of as many females as possible. Sperm production is usually cheap (Bateman 1948, Trivers 1972) and hence does not typically set limits to male reproductive success. However, as Dewsbury (1982) points out, sperm arrives in an ejaculate which may well be energetically and physiologically 'expensive' to produce. This may be because the ejaculate has to serve a nutritive function, as in some insects (reviewed in Thornhill 1976 and Marshall and McNeil 1989) or simply to fulfill mechanical requirements of sperm transfer. The latter is true in the case of the smooth newt (see below). It has been shown that the production of spermatophores is limited over a short period of time (one to two days), in smooth newts, and it seems likely that glandular depletion limits spermatophore production (Verrell 1986c).

I will argue that maximum spermatophore production over a short time period is an indirect measure of male reproductive success and is under strong selection pressure for a number of reasons. Firstly, there is a narrow window of mating opportunity, on a daily and seasonal basis. Most mating activity occurs during the evening, at dusk (Griffiths 1985, pers. obs.) limiting male mating opportunity to a narrow portion of each day. Also, peak female receptivity seems to be synchronous, with most courtship behaviour occurring over a short period immediately prior to oviposition (Verrell and McCabe 1988), hence a male that is able to produce many spermatophores over a short space of time will be at a reproductive advantage. Secondly, the mechanics of spermatophore

transfer will select for multiple spermatophore production. In the family Salamandridae, fertilization is internal, but sperm transfer occurs via male deposition of a spermatophore, which consists of a sperm cap, mounted on a gelatinous base. This sperm cap is picked up by the cloaca of the female. Manouvering the female over the spermatophore and successful spermatophore pick-up is achieved via a stereotyped courtship sequence which has been described in detail (Halliday 1974,1975). However, spermatophore transfer is not perfect (less than 50% of spermatophores deposited are actually picked up by females, even in the favourable conditions of aquaria [Halliday 1974]) and production of large numbers of spermatophores over a short period of time might be expected to be favoured. In addition, in a situation where a male deposits more than one spermatophore, a female is more likely to pick up spermatophores which are produced later in a sequence (Halliday 1974), although this effect is not always recorded (Charlotte Hosie, pers. comm.). Males that are able to deposit a large number of spermatophores tend to perform bouts of longer duration (Halliday 1976), which in turn is more likely to stimulate a female to a state of receptivity (Teyssedre and Halliday 1986). The observations of Bell (1977) and Hosie (pers. comm.) suggest that female smooth newts may accept many spermatophores, a situation likely to lead to sperm competition. Multiple paternity has been has found in the congeneric *Triturus alpestris* Rafínski (1981). Although it is still speculative at this stage, it is suggested that multiple spermatophore production could be selected by sperm competition in the smooth newt.

Body size effects on spermatophore production have been found in field crickets (*Gryllus bimaculatus*). Larger males have a shorter refractory period between matings, possibly as a consequence of the fact that smaller males invested proportionately more (in terms of weight) in each spermatophore (Simmons 1988).

The following experiments tested 66 males for the number of spermatophores they were able to produce during one manipulated encounter. 43 were tested in 1988 (with the help of C. Hosie, as part of a collaborative study) and 23 in 1989.

Method

Capture and maintenance of animals 28 males were caught as they moved towards a pond in Milton Keynes (Great Linford), 13-3-88, 16 males were captured with the aid of torch and net from another pond in Milton Keynes (Stony Stratford), 24/25-3-88 and 20 males were captured by T. Halliday in newt traps at a pond in Oxford between 24-3-88 and 7-4-88. The terrestrially caught males were housed separately in plastic aquaria (39 x 20 x 25 cm) to insure that no male elicited spermatophore deposition by other males (when male *Triturus vulgaris* are housed together spermatophores are occasionally seen adhering to individuals, pers. obs.) with gravel substrate and weed cover. *Tubifex* was provided as food. The aquatic males were placed in aquaria (60 x 45 x 25 cm), with less than ten animals per aquarium. At least two days were allowed to elapse before testing males, to allow terrestrial males to develop secondary sexual characters or to allow aquatic males to replenish any spermatophores that may have been depleted during courtship prior to capture. Verrell (1986) found that sperm replenishment occurred within 48 hrs.

Experimental procedure It has been found that male smooth newts will court an anaesthetized female presented in a 'strait-jacket' (see Halliday 1975 for details). The response of the female can thus be controlled by the experimenter. The current study presented each male with a positively responsive model female so that any limitations to male courtship were intrinsic to the male itself. This method closely follows the standard courtship encounter used by Verrell (1986c).

Each male was tested separately. The male was placed in an observation tank (60 x 37.5 x 37.5 cm) with a water temperature of 20°C +/-1°C). All newts were allowed to 'settle' in the observation tanks, since, after handling and on introduction to a new tank newts tend to show flight reaction and swim around very fast. However, in a matter of minutes they come to rest on the bottom, usually after surfacing for air at least once. At this point they can be presumed to have 'settled'. A female was anaesthetized in a 1:1000 solution of MS-222 (Sandoz) and was presented by lowering her in front of the male.

The stop clock was started when the male moved or oriented towards the female. Most males that were to deposit spermatophores were very quick to respond. The model was then operated in such a way as to mimic the behaviour of a positively responsive female, by constantly moving towards the male and, later, touching the tail during the male 'quiver' behaviour in order to elicit spermatophore deposition (see Halliday 1975). The number of spermatophores deposited (spermatophore score) was recorded. Responsive males were tested to spermatophore exhaustion. The criterion that was used to define exhaustion was that a male did not deposit for two minutes after his last deposition or last breathing ascent, which usually followed each deposition. Exhaustion was also characterised by behavioural changes; males did not display, except to remain in the wave position, and tended to sniff the female, or even the substrate, as if searching for food. If a male did not court the model five minutes after presentation, then the trial was abandoned.

Results 43 out of the 64 males tested responded to the model, depositing between one and eight spermatophores. Number of spermatophores deposited is plotted against male SVL (see Fig. 3.11). Simple linear regression of number of spermatophores on SVL shows a positive and significant relationship ($r = 0.31$, 42 d.f., $p < 0.05$). Male body size is positively related to the ability to produce spermatophores. However, this must be regarded as a weak relationship, since variation in male SVL only explains 9.6% of variance in the number of spermatophores deposited.

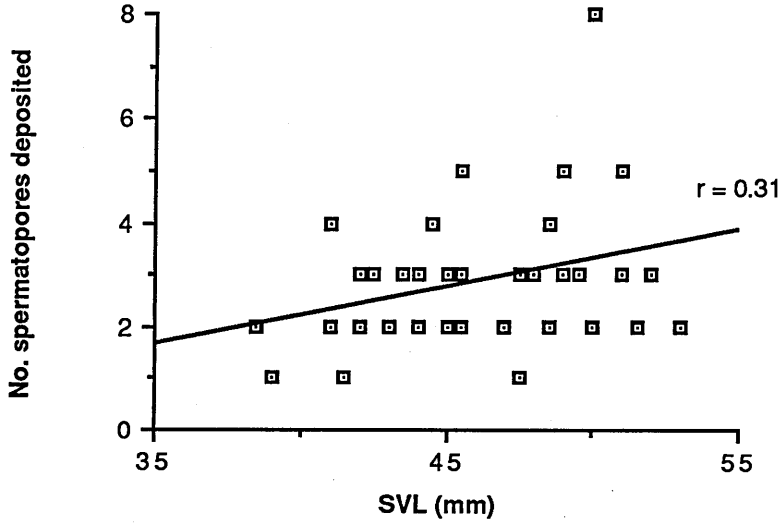


Fig. 3.11. Male body size plotted against spermatophore score (1988). Male body size given as snout-vent length in mm. Each point represents the spermatophore score for a single male in a single, manipulated courtship encounter.

Discussion The results of this study show that body size is related to the ability to produce spermatophores, but the relationship must be regarded as weak because there is a large amount of variance in spermatophore production that is not attributable to male body size (Fig 3.11). There are three possible non-size related factors that may be contributing to such large variance. Differential 'resource-accruing' abilities or differential allocation of resources may affect male ability to produce spermatophores in a manner analagous to the hypotheses given to explain the non-body size related variance in female clutch size (see Section 2.1).

Thirdly, differential effects of capture and laboratory maintenance of newts may affect the performance of males. These effects have not been quantified in smooth newts, but it is clear from work conducted during the course of this project, and other sources, that removing newts from the natural situation and maintaining them under captive conditions can inhibit male sexual behaviour (*Taricha granulosa* [Boyd and Moore 1990]) and even confining them within a natural pond, can cause males to lose reproductive condition (*T. cristatus* [Hedlund 1990]). In smooth newts the secondary sexual characters of males diminish and they become less willing to court. In both sexes animals tend to loose characters associated with the aquatic phase (see Section 3.1 for a discussion of this effect in females). Tail fins regress, the skin changes texture and some individuals try to leave the water or float around at the water surface. These effects were evident when males were maintained for longer periods than in the present experiment. Notably, some of the results in the present experiment were salvaged from a 'recovery time' experiment. This experiment was an investigation into the effect of male body size on the ability of individuals to replenish their supply of spermatophores available for deposition. Verrell (1986) found that males took between 24 and 48 hours to completely replenish their supply of spermatophores, so an experiment was devised to exhaust males of different body sizes and then record the number of spermatophores deposited at intervals of 24 and 26 hours to test the experimental hypothesis that larger males were able to recover faster. During the course of this experiment, signs of animals losing

condition became evident. The majority of the males used in the current experiment were only held in aquaria for two days prior to testing and so did not appear to have lost any condition, however it is quite possible that animals in better condition would yield different results. It is predicted that the same body size effect would emerge, but the amount of variance may decrease.

So if one, or a combination of two of the first and second alternative explanations was true, then body size may not explain variance in reproductive success in *Triturus* which would be in accordance with Malacarne and Cortassa's (1983) laboratory result that male body size (weight) in *Triturus carnifex* was not correlated with either the number of spermatophores deposited or picked up.

3.3 Body Size and Short-Term Limitation to Male Mating Capacity in the Smooth Newt (1989 Study).

Introduction The 1988 study of male body size and spermatophore production (Section 3.2) showed that although there was a significant positive relationship between male SVL and spermatophore score, there was still much unexplained variance. It was speculated that some of this variance may have been produced due to stress caused by captive conditions acting differentially on individuals and hence affecting spermatophore production. So, in the 1989 season a different approach to housing study newts was employed - namely that the newts were maintained in tanks outside to expose them to natural conditions. A repeat of the 1988 work also allowed two further lines of investigation to be followed; the relationship between spermatophore production and development of secondary sexual characters, and the relationship between body size and the temporal pattern of breathing ascents.

Newts of the genus *Triturus* show a high degree of sexual dimorphism (Halliday 1977). During the breeding season males develop dorsal crests, tail filaments and bright colouration. Darwin (1874 pp 344-345) noted the secondary sexual characters of male newts as an example of characters that could have evolved as a consequence of sexual selection and speculated that the crest of *Triturus vulgaris* is ornamental and selected through female choice. Halliday (1977) also comments that since the mating behaviour of this genus involves no amplexus this system would be an ideal situation for the expression of female mate choice. However it is not always easy to demonstrate that females choose mates on the basis of male secondary sexual characters. It is also debatable whether secondary sexual characters reflect differences in the quality of males ('good genes' argument) or whether heritability of an attractive character is in itself a strong enough feature to drive natural selection ('nonadaptive' argument) (see Kirkpatrick 1987 for further discussion). The ability to be able to produce multiple spermatophores is likely to be strongly related to male fitness via sexual selection, and multiple

spermatophore production is also likely to reflect male vigour, since spermatophore production does seem to be physiologically 'expensive'. If crest size is positively correlated with spermatophore production then male crest size may signal vigour, falling into the category of what Andersson (1986) terms condition-dependent ornaments. If, however, spermatophore production and development of secondary sexual characters are in no way related, then secondary sexual characters may represent a trait that is attractive to females irrespective of general condition and survival abilities of the male. The present study examines the relationship between male secondary sexual characters and the ability to produce spermatophores under standardized test conditions, testing a second hypothesis that the degree of development of secondary sexual characters indicates the potential mating capacity of male smooth newts.

The third line of investigation was to examine the effect of male body size on breathing. Section 2.3 outlines the consequences of surfacing to breathe during the courtship of smooth newts. From such arguments a third hypothesis was tested, that larger males would have to interrupt a courtship encounter, to breathe, earlier than a small male.

The present experiment tested 23 male smooth newts for the number of spermatophores that they were able to deposit during a manipulated encounter.

Method 58 male smooth newts were captured as they migrated towards two breeding ponds in Milton Keynes and toe-clipped to indicate date of capture (one toe-clip per newt). The newts were maintained in holding tanks outside, at a site (0.3 and 6.6 km from the ponds), in order to expose the newts to as natural conditions as possible. All males were housed in three 120 x 37.5 x 37.5 cm aquaria filled to a depth of 25 cm with water. These aquaria were in turn placed in a large tub of water, so that the water in the holding aquaria experienced a range and fluctuation of temperature that would approximate to those of the natural breeding pond (see Fig. 3.1). Similarly the newts would also experience a natural light-dark cycle. The latter is suspected to be particularly important since, during the aquatic phase, smooth newts are mainly active at dawn and dusk (Griffiths 1985). The tanks were furnished with clumps of weed (*Ceratophyllum demersum*) and pieces of broken clay pipe as refuges for the newts. Newts were provided with zooplankton (mainly *Daphnia* species) and *Tubifex* to allow feeding on an *ad libitum* basis.

Experimental procedure This was similar to the 1988 study except that newts were screened for receptivity by presenting them with a strait-jacketed female in the holding aquaria. Responsive newts were netted and removed to a test aquarium in a nearby shed. Timings of each ascent to breathe were recorded during each courtship, with time zero being established as in the 1988 study. The temperature of the water in the test aquaria ranged from 9.5-11^{o}C which was similar to that of the holding tanks at the time of testing (differing by 1^{o}C at the most). The cut-off time used as the exhaustion criterion was three minutes rather than the two minutes of the previous experiment, since males seemed slower to respond at these low temperatures.

After exhaustion each male was anaesthetized in a 1:1000 solution of MS-222 (Sandoz) and the following measurements taken:

Total length (TL): length from nose tip to tail tip (mm).

Snout-vent length (SVL): length from nose tip to hind most point of cloaca (mm).

Weight (Wt.): newts were dried on a paper towel and weighed to the nearest 10 mg.

Tail height (TH): distance between upper and lower edges of tail at maximal width (mm).

Total length, snout-vent length and weight were used as measurements of body size and tail height was used as a measure of development of secondary sexual characters. Weight condition of each male was also considered as a parameter that may affect spermatophore production. Weight condition is a measure of 'fatness', and was obtained as follows: a linear regression of weight on SVL was performed to define a relationship between SVL and weight for all of the males tested. The residuals of this regression (individual deviation in weight from this relationship) were considered to be an index of weight condition of each male.

Time, in days, that had elapsed since initial capture (days in water), and the number of days that had elapsed since the first male produced any spermatophores (days into season) were also noted for each responsive male. To compare the morphometrics of males used in this study with those of newts in a natural situation, 43 male smooth newts were captured at one of the breeding ponds, by the use of funnel traps. The same morphometric measurements were taken for these newts. These newts will be referred to as 'pond' newts and the males that courted a strait-jacketed female as 'experimental'. All pond newts were captured over the same time period that the courtship encounters were staged (26-3-89 to 14-4-89).

Crest height may seem to be the most obvious index of development of secondary sexual characters, but in practice this is difficult to measure because it becomes difficult to judge at which point the dorsal surface actually becomes 'crest', particularly in individuals with very well-developed crests. Tail height is a more discrete feature and correlates with crest height ($r = 0.824$, $p < 0.001$) for measurements made on the 43 pond newts of the present study.

Results Newts produced between two and five spermatophores. Pearson product-moment correlations were performed on each male measurement and the number of spermatophores produced (see Table 3.2). Although all correlations are positive, there is no significant relationship between any of the measures of body size (TL, SVL and Wt.) or Wt. condition and spermatophore score. TH and both measures of time produced significant positive correlations with spermatophore score ($r = 0.45$, $p < 0.05$ for TH and $r = 0.64$, $p < 0.01$ and $r = 0.72$, $p < 0.01$ for days into season and days in water respectively). However, since TH tended to increase with time, the correlation between TH and spermatophore score may reflect the confounding variable of time. When the effects of time are held constant, the correlation coefficients for TH and spermatophore score drop to 0.26 (days into season partialled out) and 0.31 (days in water partialled out). Hence there is no relationship between spermatophore score and TH when the effect of time is removed.

Parameter	r	p
Total length	0.09	> 0.05
Snout-vent length	0.11	> 0.05
Weight	0.27	> 0.05
Weight condition	0.39	> 0.05
Tail height	0.45	< 0.05
Days in season	0.64	< 0.01
Days in water	0.72	< 0.01

Table 3.2 Pearson product-moment correlation coefficients for number of spermatophores produced during one manipulated encounter and various male measurements.

Body size was not related to the timing of the first breath, but there was a significant positive correlation between body size and the number of breaths taken prior to attainment of the exhaustion criteria (Table 3.3). Hence, although small body size did not seem to allow males to prolong the length of time in courtship, smaller males overall

took fewer ascents to the surface than larger males (Table 3.3).

Variable 1	Variable 2	r	p
SVL	Time to first breath	0.18	0.425
SVL	Number of breaths	0.50	0.015*

Table 3.3. Pearson product-moment correlation coefficients to show relationship between male body size and breathing ascents. * = Significance at 5% level.

Comparison between experimental and pond newts The newts that responded to the strait-jacketed females during the course of the study were similar in size to the newts trapped in the pond (mean SVL = 47.2 and 48.0 mm respectively, $t = 1.013$, $p = 0.315$, 64 d.f.). However, the mean TH of the experimentals was actually less than that of the pond newts (11.3 and 13.1 mm, $t = 3.956$, $p < 0.001$, 64 d.f.). The reason for this is unclear. Difference in nutritional state is unlikely, since both groups had very similar weights (2.76 g and 2.69 g , $t = 0.511$, $p = 0.611$, 64 d.f.). TH was plotted against SVL on a log-log plot to examine the relationship between body size and size of male ornaments. Log TH is positively correlated with log SVL in the pond group ($r = 0.60$, $p < 0.01$), but not in the experimental group ($r = -0.14$, $p > 0.05$) (see Fig. 3.10).

		'Pond'	'Experimental'	
SVL	mean	48.0	47.2	$t = 1.013$, $p = 0.315$
(mm)	s.d.	3.64	2.64	
	n	43	23	
TH	mean	13.1	11.3	$t = 3.956$, $p < 0.001$
(mm)	s.d.	1.89	1.54	
	n	43	23	
Weight	mean	2.76	2.69	$t = 0.511$, $p = 0.611$
(g)	s.d.	0.598	0.453	
	n	43	23	

Table 3.4. Summary of body size (SVL), development of secondary sexual characters (TH) and weight for 'pond' and 'experimental' groups.

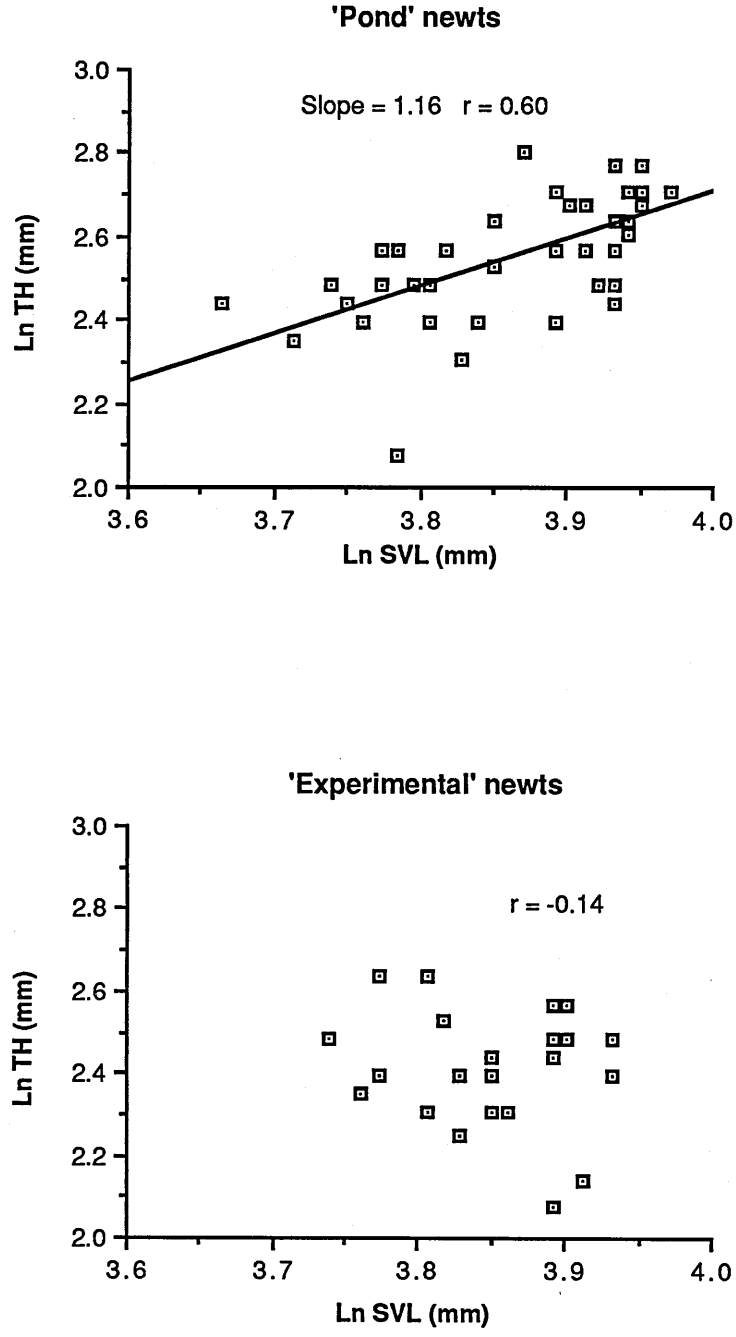


Fig. 3.12. Log-log plots to show relationship between male body size (SVL) and size of secondary sexual character (TH) for experimental and pond groups.

Discussion The result that there are only weak, insignificant relationships between any of the three measures of male body size and spermatophore number suggests that male body size has little effect on the short term mating capacity of smooth newts. The present study uses short-term mating success as a measure of reproductive success, which is open to criticism on the grounds that this measure may not reflect lifetime mating success (Banks and Thompson 1985, Clutton-Brock 1988). Considering a new hypothesis, namely that larger male smooth newts have a higher lifetime mating success than small males, it is proposed that an effect may be generated in one, or both, of two ways. Firstly, large body size is associated with greater longevity (Boddington 1978), so the lifespan of large males may encompass more mating opportunities than are available to small males. In *Coenagrion puella*, Banks and Thompson (1985) found that larger males tended to live longer, and longevity was associated with increased mating opportunities, although the relationship between body size and mating success was not significant. Secondly, larger males may simply achieve a greater mating success rate over a similar span of time, a whole breeding season for example, but this difference would not be detectable using a short-term measure of mating success such as that used in the present study. Spermatophore production over a whole season is currently being investigated at the Open University by Tim Halliday and Verina Waights.

There is a trend, although insignificant, that suggests that there may be some effect of weight condition on spermatophore production. The trend suggests that males in better condition can produce more spermatophores, which is an appealing conclusion, since better condition males are those that have presumably accrued more resources in terms of food, and hence should be better able to cope with the demands of spermatophore production. This relationship may be worth further study.

The factors that significantly correlate with male spermatophore score are TH, days into season and length of time in the water. Length of time in water proved to be a slightly better predictor of spermatophore number ($r = 0.72$) than days into the season ($r = 0.64$). This makes it tempting to suggest that male smooth newts mature their spermatophore

supply in the water rather than purely as a consequence of season. However, larger sample sizes would be needed to ascertain whether this is in fact the case. The significant positive correlation between TH and spermatophore score could then represent the relationship between the size of the secondary sexual characters and short term mating ability. This would fit the theory of the good genes argument since according to this school of thought, females will tend to mate with the most vigorous or viable males. However, the element of time must also be considered. Individuals tested later in the study not only produced more spermatophores but also had larger tail heights. A regression analysis of TH on days into season and time in water shows a significant positive relationship in the former case ($r = 0.42$, $p < 0.05$) and a positive but not significant trend in the latter ($r = 0.34$, $p > 0.05$), which suggests the secondary sexual characters were gradually developing as time progressed. This pattern is to be expected, since Griffiths and Mylotte (1988) showed that crest heights of smooth newts at a site in Mid-Wales gradually increased during March and April, reaching a maximum in mid-May. Spermatophore scores increase with increasing TH, but both of these parameters are increasing as a function of time, since the effect of holding time constant is to reduce the partial correlation coefficients of TH and spermatophore to below the 0.05 level of significance. So, THs of a sample of male newts, measured at any particular time during the course of their breeding season, do not signal the potential number of spermatophores that individuals could produce.

Verrell (1986c) examined male mating capacity using a similar procedure to the present study. His results are also similar to the present study in that males produced between one and six spermatophores in response to a strait-jacketed female. However, he differs in interpretation of these results. He presented a strait-jacketed female to a male in the presence of three other females. After the male completed an encounter (ie. produced no more spermatophores) a new strait-jacketed female was presented to the male with the result that only 9% of his males went on to produce another spermatophore. Verrell's conclusion was that male *T. vulgaris* is unable to exploit a multi-female situation by engaging in multiple matings. Nevertheless, he also notes that the strait-jacketed female must represent a 'super-responsive' mate. This creates an unnatural mating situation.

Unrestrained female *T. vulgaris* do not normally respond as quickly or as positively as a strait-jacketed model. In the laboratory situation they rarely elicit more than three spermatophores (Halliday 1974). In the field, most courtship encounters do not proceed to exhaustion but are terminated by the female swimming away (Verrell and McCabe 1988). The conclusion I draw from combining results of 'strait-jacket studies' and results obtained from unrestrained newts is that males can produce many spermatophores (between one and six) within a short period of time (for example one evening of courtship activity) and that in a natural situation this can potentially lead to the insemination of more than one female, rather than to the repeated insemination of a single female.

To reverse the implicit assumption of the first experimental hypothesis, that larger males can produce more spermatophores, it could be asked why smaller males should not be able to produce as many spermatophores as larger males? Smaller males possess smaller testes (Verrell and Francillon 1986) but they could simply produce smaller spermatophores, as *Desmognathus ochrophaeus* seems to do (Houck obs. in Houck and Francillon-Vieillot 1988). However, there may be an optimum or critical minimum size for spermatophores. Spermatophores are picked up by females by adhering to the cloaca. If spermatophore size has an effect on pick-up success it is likely that the height of the spermatophore above the substrate (or the total height of the spermatophore and its base) is the critical factor. A female may walk over a small spermatophore without touching it. If this were the case, then smaller males would have to produce larger spermatophores relative to body size that a larger male and hence not be able to produce as many of them. For example, if it is assumed that the optimum/critical spermatophore height for *Triturus vulgaris* is 5 mm, then a big and small newt (SVL within the range found in Milton Keynes animals) must both produce a spermatophore of the same height.

	SVL (mm)	Sppt. height (mm)	Ratio	Volumetric ratio
Big	50	5	10:1	1000:1
Small	40	5	8:1	512:1

So one spermatophore would be related to the body volume of a big newt by a factor of 1/1000 and the volume of a small newt by a factor of 1/512, hence smaller newts are producing relatively very much larger spermatophores. However this does not explain the phenomenon of smaller species or sub-species producing fewer spermatophores than larger congenerics. In this latter case, if there was an optimum spermatophore height relative to SVL, then spermatophore volume would decrease at the same rate as body volume.

It should be noted that the present study deals with the short term mating capacity of smooth newts, arguing that the production of many spermatophores over a short period of time should be strongly selected. This must be viewed from a wider evolutionary perspective. Why is spermatophore production in smooth newts limited to between one and six over a short period of time? Arnold (1977) reviews studies of five ambystomatid species which have mean spermatophore scores of between 12 and 40 per night and yet *Desmognathus ochrophaeus* generally deposits only one spermatophore every two to three nights, even in the presence of two females (Verrell 1988). The most obvious explanation for these large difference lies in the differences between the temporal mating patterns of these species. Arnold predicts that the length of courtship season will affect the spermatophore production patterns of males. *Desmognathus ochrophaeus* has a prolonged breeding season from September to June, whereas ambystomatids exhibit a more explosive type of breeding. *T. vulgaris* has a breeding season somewhere in between these two extremes (March-May) and also seems to exhibit an intermediate capacity for spermatophore production. The length of reproductive period of *T. vulgaris* would be expected to select males that are able to inseminate females at any time within that breeding season. Smooth newts do seem to fit this prediction, being able to produce spermatophores over long periods of time (Halliday 1976). So the results of this short-term study must be treated with caution since the optimum spermatophore strategy for smooth newts would seem to to be able to produce as many spermatophores as possible to optimize on any periods of multiple female availability and yet not to deplete

spermatophore supply to the extent that an individual male is not able to inseminate any females that are receptive later in the season. The relationship between short-term spermatophore production and total seasonal production is unknown, as is the effect of body size on seasonal production.

The third hypothesis, that larger males should have to breathe and hence interrupt a courtship encounter earlier than a small male was rejected since there was no relationship between this event and body size. However, it was noted that small males actually surfaced to breathe on fewer occasions than large males during the courtship encounters. The significance of this in a natural setting is not obvious. Loss of a female due to the need to ascend to breathe is likely to occur at the first ascent. Large males can stay down for just as long as small males, so small males will have no advantage. However, the need for larger males to make more ascents during periods of intense activity (courtship) may mean that during periods of multiple female availability they may in fact suffer greater losses of females than small males.

Comparison between experimental and pond newts The differences recorded between the development of the secondary sexual characters of the two groups must be linked to some aspect of maintaining these animals under captive conditions. Differences in nutritional state seem unlikely, since live prey items were available on an *ad libitum* basis and there was no difference in weight between the two groups, but since the experimental newts did not receive such a wide variety of prey items as are present in a pond, it is possible that the pond newts obtained a better quality diet. Other differences between the two environments are the high density of males and the lack of exposure to females experienced by the experimental group. How this will affect the development of secondary sexual characters is unclear. Another possible explanation is that males may come into peak breeding condition at different times. The newts that were captured in the pond were probably actively seeking females (the traps were set in the shallows, at the edge of the pond - areas only frequented by courting or ovipositing newts) whereas the experimentals were presented with a female on a purely random basis, not determined by the degree of development of secondary sexual characters. This difference in the methods of sampling

the two groups may have affected the THs recorded.

One other piece of information revealed from the morphometrics of the pond group is that there are significant positive correlations between SVL and crest height ($r = 0.60$ significant at 0.05 level, for log-transformed data and see Fig. 3.12). This agrees with the trend found by Hedlund (1990) for *T. cristatus*, but differs from the findings of Griffiths and Mylotte (1988) for *T. vulgaris*. They recorded measurements from samples of 12 smooth newts taken at intervals of 14 days over the aquatic period. They found only a weak relationship between SVL and secondary sexual characters in *T. vulgaris* (toe flap width and CH) and so reasoned that if development of these features is related to mating success then mating success cannot be related to male body size. The difference between the two sets of findings may be due to sample size, since there is large amount of variance in TH for any particular SVL, which may have made it difficult to observe any trends using sample sizes of 12. Breaking down my data for the first 36 newts captured, into three groups of 12 males does affect the significance of the resulting correlation coefficients. The results of such an analysis are shown in Figure 3.13 using a similar format to Griffiths and Mylotte (1988). All correlations are positive, but two of the SVL-CH correlations and one of SVL-TH correlations are not significant at the 0.05 level.

Hence it is concluded that there is a significant positive correlation between male SVL and the size of the secondary sexual characters. It is also evident that there is a tendency for larger males to have relatively larger epigamic characters. To illustrate this TH has been plotted against SVL on a log-log plot (Fig. 3.12). The gradient of the regression line for the pond newts is 1.16, which indicates that TH increases at a faster rate than SVL. However, this is not statistically significant ($t = 0.757$, which is a lower value than that needed to attain significance at the 5% level for 40 d.f., 2.021). However, in terms of real newts this translates to an increase in SVL across the range of 40 to 50 mm (25% increase) being accompanied by a 30% increase in TH.

There is evidence to suggest that female newts do choose males on the basis of secondary sexual characters. Malacarne and Cortassa's (1983) work demonstrated that, in

laboratory courtships, male *T. carnifex* with larger TH deposited more spermatophores and females tended to pick up more spermatophores from males that had a larger TH, and Hedlund's (1990) work on *T. cristatus* provides evidence that females preferentially pick up spermatophores from males with higher crests. Female mate choice on the basis of crest size has also been demonstrated in *T. vulgaris* (C. Hosie, pers. comm.). Hence, large male body size may be selected because larger males have larger crests which make them more attractive to females. Note that the problems of distinguishing female mate choice from other confounding variables are discussed in Section 3.3.

Conclusion Male body size was not related to two aspects of short-term mating capacity. First, male spermatophore production was not related to body size, although there was a tendency for weight condition to be positively associated with spermatophore score. Second, smaller males were not able to delay the time before they needed to surface to breathe. Tail height was not related to short-term mating capacity.

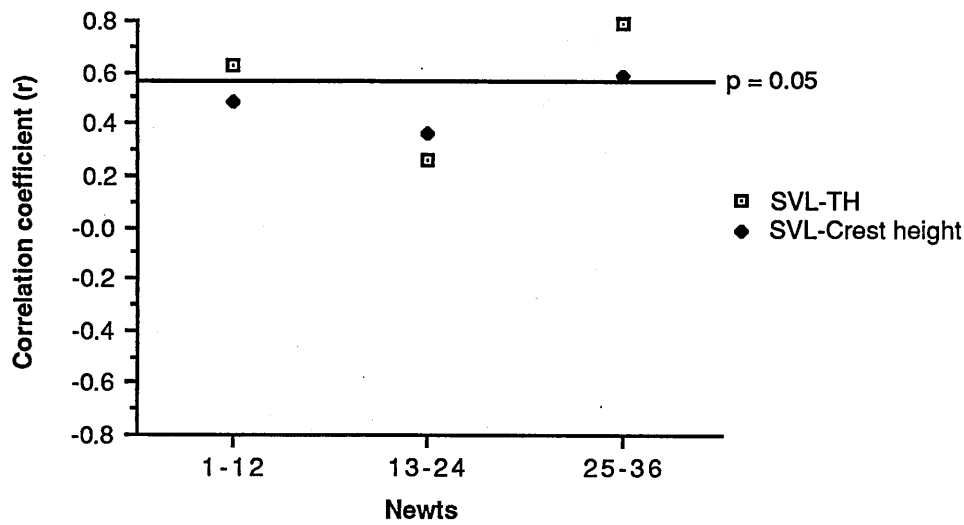


Fig. 3.13. Presentation of data for the first 36 pond newts captured, in a manner similar to that of Griffiths and Mylotte (1988) ie. analysis of data divided into three samples of twelve newts each.

Chapter 4
Larval Growth

There is little quantitative information on the growth of *Triturus* larvae, but what is known is summarized below:

1. Growth rate The larval stage of the life cycle is the period of fastest growth (see Chapter 1).

2. Shape of growth curve Bell and Lawton (1975) measured the larvae of *Triturus vulgaris vulgaris* in natural and laboratory populations. They concluded that size increase (total length) was rectilinear.

3. Variance in larval period Bell and Lawton also found that larvae grew in three size cohorts, generated by three peaks in female oviposition (see Chapter 3.1.4). This cohort structure seems to have been preserved throughout the larval period since Bell (1977) found that metamorphs left the pond in two waves, one in August and one in September. However, such peaks of female oviposition and eft emergence may equally well be caused by variation in weather patterns. Oviposition is affected by temperature (Section 3.1) and movement of recently transformed amphibians away from their larval ponds seems to occur on warm, wet nights (pers. obs.). Verrell (1985a) notes a different pattern of larval growth. Within one pond he found evidence of synchronized oviposition (May-June) but variable larval emergence (Aug-Dec, a period of some 17 weeks). The same pond is also known to produce some larvae that overwinter, so larval period may, in fact, be spread over a year or so. Harrison (1985) also found variable larval emergence from a single pond. The Bell and Lawton account of larval growth in smooth newts does not exhibit the variance in larval period that is typical of amphibian life histories, whereas Harrison's and Verrell's findings are more compatible with the usual pattern. Variation in larval development and growth rate is also recorded in natural populations of anuran larvae, and has also been shown to persist when individuals are reared individually under identical conditions (Travis 1981). In the laboratory, using techniques of *in vitro* fertilization to

obtain controlled matings, he was able to demonstrate that there are both maternal and genetic factors controlling larval growth rate and larval period in *Pseudacris triseriata.*

Urodeles of the genus *Triturus* show an even more complex picture than most amphibians studied, by virtue of the fact that the females oviposit eggs singly and over a prolonged period. So as well as intrinsic variation in larval growth rate, individual newt larvae may experience different growth conditions due to differential timing of oviposition , generating even more variance. This will produce a cohort containing individuals of different developmental stages, experiencing different environmental conditions at comparable developmental stages.

4.1 Individual Fitness Consequences Mediated Through Larval Growth

It is thought that growth during the larval stage may affect adult body size and hence fitness (see Chapter 2). Data obtained by monitoring natural populations of two anuran and one ambystomatid urodele species have shown that larval growth responses can affect adult body size. Berven and Gill (1983) marked cohorts of *Rana sylvatica* metamorphs and recorded their later recruitment into the adult population. They found that timing and size of transformation can affect adult body size and survivorship. Early metamorphs had higher survivorship than late metamorphs, and larger metamorphs had lower mortality than smaller individuals and tended to mature as larger adults. Smith (1987) monitored *Pseudacris triseriata* in a similar manner, and found that, although body size did not affect survivorship, large and/or early metamorphs had a better chance of maturing after one year rather than two. Due to a longer pre-maturity period the small and/or late metamorphs suffered an increased chance of mortality prior to reproduction. Large metamorphs also tended to mature as large adults. Semlitsch et al. (1988) showed that size and timing of transformation affected size at first breeding in *Ambystoma talpoideum.*

Experimental manipulation of the larval growth environment can also generate larval growth responses that affect adult body size. Semlitsch (1987) reared *Ambystoma talpoideum* paedomorphs under different regimes of density, 'pond drying' and food. All

three factors affected larval growth and adult body size. However, it may be unwise to generalize this result to species that exhibit a prolonged terrestrial juvenile stage. Semlitsch's paedomorphic *A. talpoideum* matured within their first year, so that the adult and juvenile stage occur very closely in time, and occupy the same environment.

The size-related growth advantage that larger metamorphs experience during the juvenile growth phase may simply be related to the fact that they maintain their initial size superiority and so are able to mature either earlier or at a larger body size, but it may also be related to physiological advantages associated with larger size. Pough and Kamel (1984), in a comparative study, found that larger metamorphic anuran species were physiologically better equipped to cope with terrestrial activity. They predict that the same effect may exist within a species, so that small metamorphs will have a lower aerobic capacity than their larger peers. Differential aerobic performance could affect foraging success and survival.

In addition to the effects of body size at and timing of transformation on adult body size, larval growth rate may also have an important effect on survival of the larvae themselves. Faster growing larvae (1) tend to be less likely to suffer mortality due to pond desiccation, (2) are better able to escape predation, (3) in competitive situations tend to be superior to smaller larvae and (4) may escape from pond freezing. Considering these three points in greater detail:

1. It has been proposed that rapid larval growth will be selected in temporary ponds as a means of avoiding death as ponds dry out (Wilbur and Collins 1973, Wilbur 1980). This may be of relevance to newts since they frequently breed in temporary ponds. This occupation of temporary ponds may be a necessary strategy to avoid fish predation, as proposed by Heyer et al. (1975) with respect to anuran larvae. Inferential information exists to suggest that fish predation can adversely affect larval amphibian populations, because introductions of fish to upland lakes can be accompanied by the disappearance of amphibian populations (Dolmen 1982, Bradford 1989).
2. The larval stage of amphibian life cycles is subject to high mortality due to predation (Werner 1986). It also seems that in an aquatic environment, larger individuals are better

able to escape predation. This is true for fishes (Weatherley and Gill 1987, Wootton 1990) and amphibian larvae. Kusano (1981) found that bigger larvae of *Hynobius nebulosus* were more likely to escape from predation attempts by *Cynops pyrrohogaster*. Cooke (1974) found that, over a certain body size, the larvae of *Rana temporaria* became invulnerable to predation by *T. vulgaris* in experimental tanks. Heyer et al. (1975) found that, in trials, larger anuran larvae were better able to avoid predation from *Leptodactylus pentadactylus* (a predatory tadpole) and *Pantala flavescens* (an odonate) larvae. Caldwell et al. (1980) showed that in laboratory trials, larger *Hyla gratiosa* larvae were better able to escape predation by larval *A. talpoideum*. Travis et al. (1985) found that, in laboratory trials, larger *Rana areolata* were better able to escape an odonate predator. Alford (1989) found that anuran larvae were able to grow to a size at which they were invulnerable to predation by *Notopthalmus viridescens* in a cattle trough experiment.

The generally accepted explanation for this decreased vulnerability of larger individuals is that many predators can only handle prey below a certain size threshold (Weatherley and Gill 1987). This is particularly true of gape-limited predators, which swallow their prey whole and hence are limited by the aperture size of their jaws. Gape-limited predators that may attack newt larvae include fish and other newts, both adults and larger larvae. However, it is also clear that predators that do not engulf prey whole, such as odonates, may show size limitation in capture of prey items. Heyer et al. (1975) and Travis et al. (1985) showed that larger anuran larvae were better able to escape odonate predators. This brings about a second possible explanation, that escape from predation through size may be due to the better escape responses of larger (and potentially better developed) larvae. Whatever the mechanism involved, clearly the longer the period of time spent at a small size, the greater the risk of predation. Fast larval growth should minimize this risk, and Travis et al. (1985) suggest that size-related predation may exert a significant selection pressure for fast larval growth.

It should be noted that there are two further potentially confounding effects that may be relevant to the above discussion of size-dependent predation. These confounding effects both arise from the fact that larger larvae will tend to be older larvae. So, firstly,

they may have benefitted from experience of increased exposure to predators and in some way have become better at avoiding predation. Secondly, the older, larger larvae in a population may also be present by merit of their abilities to escape predation. This confounding effect of age can be removed by experiments performed on larvae of variable sizes, but similar ages, or controlling for the effect of experience. This was incorporated into the methodology of Heyer et al. (1975), Caldwell et al. (1980) and Travis et al. (1985) but was overlooked by Kusano (1981), Cooke (1984) and Alford (1989).

3. The superiority of larger larvae in competitive situations is the norm (eg. In *Rana temporaria* and *Bufo bufo* [Savage 1952], in *Rana utricularia* [Steinwascher 1978] and in *Bufo woodhousei* [Woodward 1987]). For a discussion of the mechanisms of intraspecific larval competition see Section 5.3. This size advantage may not be as universally applicable to urodele larvae as it seems to be to anurans. Smith and Petranka (1987) have shown that under certain conditions, namely when natural prey levels are low and prey items small, then large larvae of *A. jeffersoniam* were not able to assimilate as much food relative to body size as small larvae and were hence less efficient feeders.

The lack of information concerning the growth of *Triturus* larvae may be due to the fact that ova are oviposited over a prolonged period (see discussion in Chapter 3.1), meaning that there is no single-aged cohort to trace through its larval phase. To overcome this problem, in the present study, newt larvae were grown in a laboratory, under controlled conditions. The species used in the course of this project were selected according to availability and suitability for the type of work. Trends observed in results of one species are probably relevant to the other *Triturus* species, but this need not necessarily be true of all aspects.

4. In situations where ponds freeze to the bottom during the winter, fast larval growth may help larvae to escape being frozen with the ponds. In high altitude populations of *T. alpestris*, larvae are often killed in this way in the autumn (Helmut Faber and Robert Schabetsberger, pers. comm.).

4.2 General Methods For Rearing Larvae In The Laboratory The following describes the methods which were used to rear newt larvae under laboratory conditions. Any variations on these techniques are outlined under 'methods' sections of specific rearing studies or experiments.

A note on staging Staging refers to the process of describing and numbering the developmental stages of amphibian larvae as an aid to reference. Some authors only describe the egg stages eg: Epperlein and Juninger (1982) only give 37 stages up to the feeding stage (for *T. alpestris*). Three other staging series were considered: Glaesner (1925), Gallien and Durocher (1957), Gallien and Bidaud (1959). All three of these series are essentially very similar, the latter two being based on Glaesner's series. The one that will be referred to for the purposes of this thesis is Gallien and Bidaud (1959), since this is more easily obtainable than Glaesner's. Gallien and Durocher is equally useful and is compared with Gallien and Bidaud in the Table 4.1 below.

Collection of ova Ova were collected from females maintained in aquaria due to the difficulty in finding ova in natural ponds. This had the advantage of standardizing the conditions under which ova were maintained prior to any rearing study. However it suffers from the disadvantage associated with maintaining newts in captivity in that they lose reproductive condition (see Sections 3.1.2 and 3.1.4) and hence tend to produce very few ova.

Females were collected from three ponds in the Borough of Milton Keynes. Females were housed in glass aquaria (60 x 45 x 25 cm) with a gravel substrate and a water depth of 15 cm. *Ceratophyllum demersum* and/or polythene 'weed' were provided on which newts could oviposit (as in Section 3.1). A photoperiod of 16:8 L:D was provided with a one hour dawn and dusk period.

Ova destined for use in rearing studies were obtained from as many different females as possible, since this project is concerned with the natural variation that is typical of a population as a whole. Ova were collected daily and removed from the substrate by gently peeling away the polythene or plant material. The ova were hatched in tissue culture dishes,

Table 4.1. Comparison of urodele staging series:

	Glaesner (1925) *Triturus vulgaris*	Gallien and Durocher (1957) *Pleurodeles waltlii*	Gallien and Bidaud (1959) *T. helveticus*
Appearance of third digit on fore foot	41	41	40
Start to capture prey	-	38	42
Appearance of fifth digit on hind foot	53	53	53
Tail fins resorbed	55	55c	55c
Gills completely resorbed	56	56	56

N.B. Stage 56 is regarded as attainment of complete transformation in the above series, but Gallien and Bidaud stage 55c is used in the present study, for reasons outlined in General Methods text.

with one ovum per cell, in an incubator cabinet at 25°C and with a 16:8 L:D photoperiod.

When recording the larval period of individuals it is important to note that *Triturus* larvae hatch at various stages of development. Hatching of *T. vulgaris* occurred from Gallien and Bidaud stages 33-40 over the course of the present project (fore-limb bud present to emergence of third digit on fore-limb). Variation in development at hatching has been recorded in other species (eg. *Ranidella signifera*, Williamson and Bull 1989). The ecological consequences of this phenomenon are not known for *Triturus* species, but Petranka et al. (1987) concluded that hatching at a later stage reduced the chance of predation by *Phagocata* (a flatworm) in *Ambystoma texanum*. Some arbitrary developmental stage was needed to standardize the first day of larval life. Gallien and Bidaud stage 40 was selected since all larvae hatch by this stage and the detection of the initiation of the development of the third digit on the fore-limb is a discrete event. This stage occurs as the larvae begin to capture prey items, although Gallien and Bidaud actually record this event as occurring at a later stage (42). Development of the larvae was monitored during and immediately after the hatching period on a daily basis to record when each larva reached stage 40 and this was used as day zero of larval life. Total length (TL) was measured at this stage, using a stereo microscope with an eyepiece graticule. Each larva was then transferred to its rearing container. These were transparent plastic beakers containing 0.2 litres of water for larvae reared singly, or larger containers for larvae reared in groups (see Chapter 5). The water used was conditioned by allowing it to stand in a large, planted aquarium and was drawn off as necessary. Unconditioned tap water does not adversely affect the larvae but it can kill off zooplankton provided as food.

The individual beakers were kept on green plastic trays. The beakers were rotated within each tray, and the tray positions themselves were rotated three times a week in order to minimize any possible positional effects.

In the natural situation *Triturus* larvae feed on a wide variety of live prey items, especially planktonic crustacea (Avery 1968, Bell 1975, Dolmen and Koksvik 1983). One study reported a switch to chironomid larvae later in ontogeny (Bell 1975). Larvae were

never seen to consume any non-living alternatives during the course of this project. This made quantification of food supply time-consuming, so as an alternative, all larvae (except those in Section 5.2) were fed on an *ad libitum* basis, checking daily that no container was ever devoid of any live prey items. The larvae were initially fed on zooplankton netted from a local pond and strained through a small hand net. As soon as the larvae had grown large enough, *Tubifex* was added to the diet. As larvae attained Gallien and Bidaud stage 53, the diet of *Tubifex* was supplimented with *Daphnia* twice a week. *Tubifex* was purchased from a commercial supplier and *Daphnia* was cultured outdoors in two plastic tubs and netted from some temporary ponds in parkland adjacent to the University campus.

Initially, larvae were measured using a stereo microscope and graticule, and as they grew larger, by placing larvae in a V-shaped trough marked with mm graduations. Total length (TL) and later, as they grew bigger, snout-vent length (SVL) were recorded to the nearest 0.5 mm. *Triturus* larvae are too small and delicate to weigh, especially during the early part of their larval lives.

Prior to metamorphosis a piece of polystyrene tile was floated on the water to allow the larvae to emerge. In the 1987 season the point of completion of metamorphosis (from now on referred to simply as 'transformation') was determined as being emergence from water. Efts did usually leave the water at this stage, but it was not found to be a reliable indicator of completion of the larval phase, since some animals complete metamorphosis but drown, whilst a few others remain aquatic for a time after completion of metamorphosis. Hence in 1988 and 1989 visual inspection of the aquatic animals as well as removal of emerged efts was used when checking for transformed individuals. The feature used to denote this stage was complete regression of the tail fin (Gallien and Bidaud stage 55c). Note that this differs from completion of metamorphosis in Gallien and Bidaud's staging. They use complete disappearance of the fins and gills as an indicator (stage 56), which does in fact mark the end of changes in external morphology, but is not matched by the ecological end of the larval phase in *Triturus vulgaris*. Efts are frequently observed in the field, leaving ponds, but still bearing traces of the gills, or 'gill scars',

whilst in the laboratory, larvae may drown before attaining complete gill resorbtion.

At transformation each eft was anaesthetized in MS-222, following the procedure outlined in Section 3.1.2 for adult news. TL and SVL were measured to the nearest 0.5 mm on a mm rule. Efts were toweled dry to remove any excess water and weighed to the nearest 10 mg. Metamorphs of native species were released at their parental ponds.

4.3 Pattern of Larval Growth The following is a descriptive account of the growth of the larvae of three species (*T. alpestris*, *T. cristatus* and *T. vulgaris*) in the laboratory. The purpose of this rearing study was to record and define the nature of the larval growth trajectory under standardized, laboratory conditions, and to quantify variance in the timing of transformation and body size at transformation. Wilbur and Collins (1973) proposed an ecological model to explain variation in timing of transformation and body size at transformation. This model assumes that larvae are in some way able to monitor their own growth rate and use this information as a measure of the quality of the larval growth environment. If growth is rapid, then they will tend to prolong the larval period, to lengthen the time spent in the fast growth environment, and transform at a large size; if growth is slow, they will leave the larval environment earlier, to escape the poor growth conditions, at a smaller body size. Under similar growth conditions, this model predicts that all newt larvae should transform at the same size and at the same time, unless there is natural variation in individual larval growth rates.

Section 4.1 outlined the advantages of fast larval growth. Hence it might be expected that there will be little genetic variability in the growth of newt larvae, which should be expressed as similar growth rates for larvae grown under identical conditions. However, there is evidence to suggest that genetic variance for growth rate does exist in ectotherms. The success of artificial selection programmes undertaken in aquaculture suggests that there is genotypic variation in the control of the growth rate of fish (Wootton 1990) and the *in vitro* fertilization work of Travis (1981) and Newman (1988) suggests that there is natural, genetic variation in the larval growth of anurans. If this variation does become manifest in

the growth of newt larvae, then the Wilbur-Collins model predicts that the faster growers will prolong larval period and transform at a larger size.

Methods Larvae were reared in individual plastic beakers as described under general methods, except for the following variations. The *T. alpestris* were kindly donated by Colin Melsom, as ova. These ova were allowed to hatch in a container (28 x 24 x 15 cm) filled to a depth of 5 cm with unconditioned tap water. Once all larvae had hatched, 24 were selected as being at the same developmental stage (Gallien and Bidaud stage 40) and measured as described in general methods. 22 of these larvae were reared to transformation, the remaining two dying prior to this event. The rearing beakers were kept on a laboratory bench. Temperature in the laboratory fluctuated between 23 and 27°C and the larvae were exposed to a natural photoperiod via the windows. Larvae were staged and measured every week.

The *T. cristatus* and *T. vulgaris* were not reared as a single aged cohort, because of the difficulty in obtaining large numbers of ova of a similar age at a given time. *T. cristatus* ova were collected, under licence from the Nature Conservancy Council, from the O.U. pond. Ova were found wrapped in the folded leaves of *Myosotis palustris*. *T. vulgaris* ova were collected as described in general methods (Section 4.2). A minimum of four females contributed ova that survived to the metamorph stage.

Larvae were reared as described in general methods, except that the *T. cristatus* larvae were reared in larger beakers, containing 1 litre of water. These larger containers were used since *T. cristatus* larvae are larger than the other two species, and it is not known if container size affects the growth of larval urodeles. Larvae were reared at a constant temperature of 20°C by placing their beakers in water baths. These water baths were three tanks (60 x 45 x 25 cm) heated to 20°C by an aquarists' heater-stat. The baths were placed in a cooled room so that ambient temperature stayed at 12°C. A photoperiod of 16:8 L:D was maintained. The beakers were rotated between baths and within the baths twice a week, to minimize any possible positional effects. Larvae were measured twice a week. 20 *T. cristatus* larvae were started, of which 18 were reared to transformation, the

remaining two died prior to this stage. 28 *T. vulgaris* larvae were started and 18 of these were reared to transformation, the remaining ten either died or were discarded after signs of developmental abnormalities.

Results

Shape of the growth curve Changes in TL for all three species are shown in Fig. 4.1. The data for *T. alpestris* are plotted as mean values at each measurement, since this sample represents a single age cohort. The data for the other two species are plotted as individual TL measurements at various ages, since these larvae were not single aged cohorts.

During the course of these rearing trials it became apparent that there was much variation in timing of transformation and body size at transformation. Descriptive data are shown in Table 4.2.

		mean	s.d.	range	n
T. alpestris	Larval period (days)	80.6	26.9	51-143	22
	TL (mm)	46.8	6.18	36-59	22
T. cristatus	Larval period (days)	76.9	6.78	69-91	18
	TL (mm)	77.5	3.94	73-87	18
T. vulgaris	Larval period (days)	50.1	7.87	41-71	16
	TL (mm)	40.4	4.16	34-49	16

Table 4.2 Body size and timing of transformation for the three species, *T. alpestris*, *T. cristatus* and *T. vulgaris*, reared in the present study.

It also became apparent that the later individuals to transform were larger than those larvae with shorter larval periods. Body size at transformation is plotted against larval period in Fig. 4.2. The data were log-transformed to normalize the otherwise positive skew; there tend to be more small, early-transforming metamorphs than large, late-transforming metamorphs. Linear regressions were performed on all three data sets, and Pearson product-moment correlation coefficients are 0.84, 0.79 and 0.80 (all significant at the 0.01 level) for *T. alpestris*, *T. cristatus* and *T. vulgaris* respectively. This size increase with time fits well with the prediction of Wilbur and Collins (1973), since the large metamorphs are those that have grown well during the larval stage, and prolonged the larval period as a consequence. However, an examination of larval growth rate and larval period does not validate this conclusion. Mean larval growth rates (calculated as change in TL per day) are in fact significantly negatively correlated with larval period (*T. alpestris* r

= -0.84, $p < 0.01$; *T. cristatus* r = -0.70, $p < 0.01$; *T. vulgaris*, r = -0.50, $p < 0.05$). See Fig. 4.3. Prolonged larval periods allowed attainment of larger body size, but were associated with a slow growth rate over the whole larval period.

According to the Wilbur-Collins (1973) model, recent growth history should be a good predictor of size at transformation. This is tested here by comparison of the growth trajectories of the early and late metamorphs. Early metamorphs are the first 50% of metamorphs to emerge for each species, and late metamorphs are the remaining 50%. If the Wilbur-Collins model has any relevance to the larvae grown in the present study, then the long larval period individuals should express faster growth at some stage during larval life. However, the growth curves of early and late metamorphs do not seem to be very different (see Fig. 4.4), except of course that the late metamorph curve is longer. The most noticable difference between these curves occurs in the case of *T. cristatus*. To establish whether there is any difference in early larval growth between these early and late metamorphs, growth rates for the two groups were compared. Early growth rates were calculated as change in TL over the first 40 days of larval life. Note that not all larvae were measured on this day, so TL and age on the nearest date to 40 days were used to calculate individual growth rates. Mean growth rates are 1.108 and 1.050 mm/day for the early and late metamorphs respectively and these means are not significantly different ($t = 2.016$, 15 d.f., $p = 0.062$) and any trend that they express is in the opposite direction to that predicted by the Wilbur-Collins model. The growth curves of early and late metamorphs for the other two species are even more similar. Hence it does not appear that growth rate during any stage of larval life affects the length of larval period.

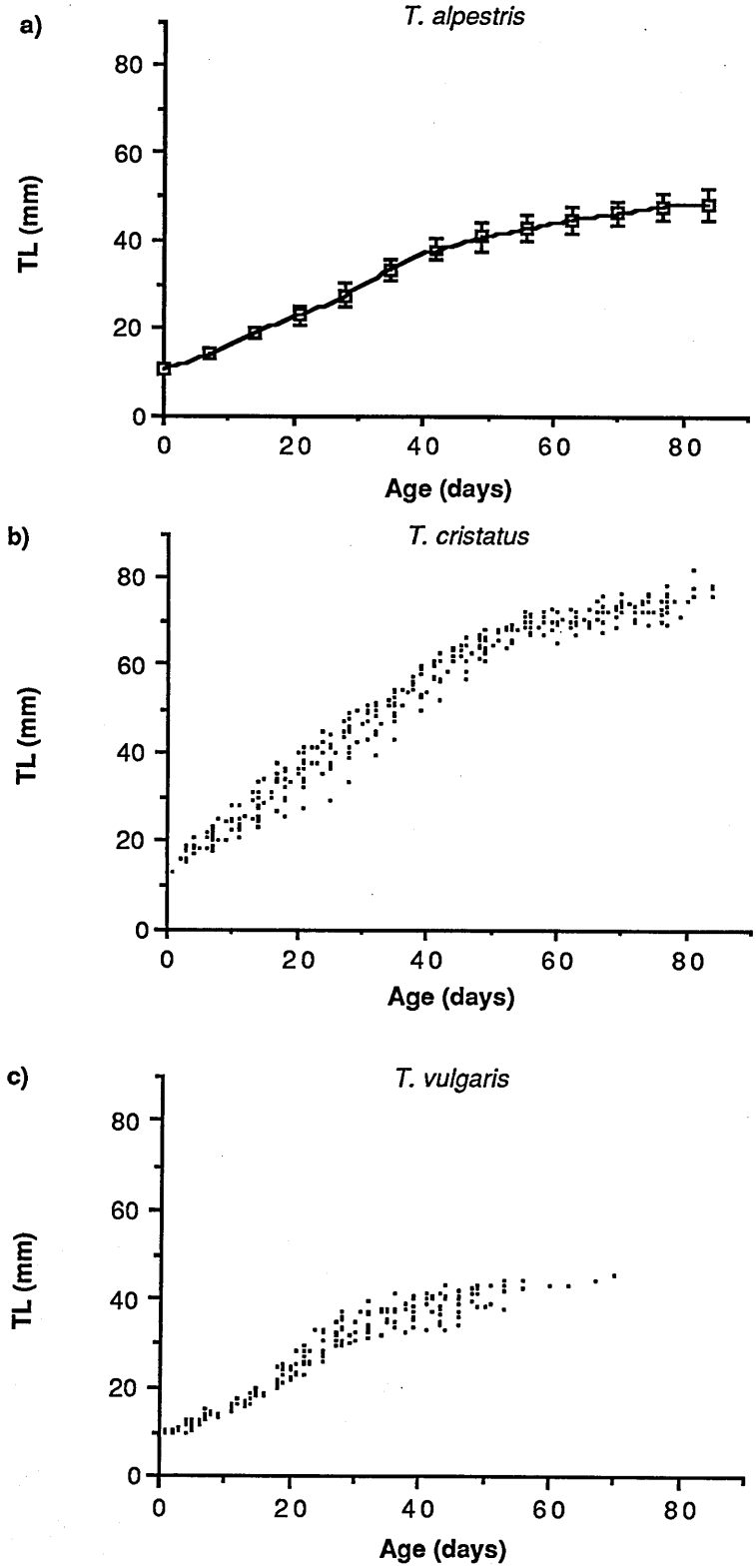


Fig 4.1. Graphs to show larval growth for three *Triturus* species under laboratory conditions. Growth is shown as TL plotted against time for (a) *T. alpestris*, (b) *T. cristatus*, (c) *T. vulgaris*. Mean values and standard deviations are given for *T. alpestris*. For *T. cristatus* and *T. vulgaris* each point represents one measurement for a single individual.

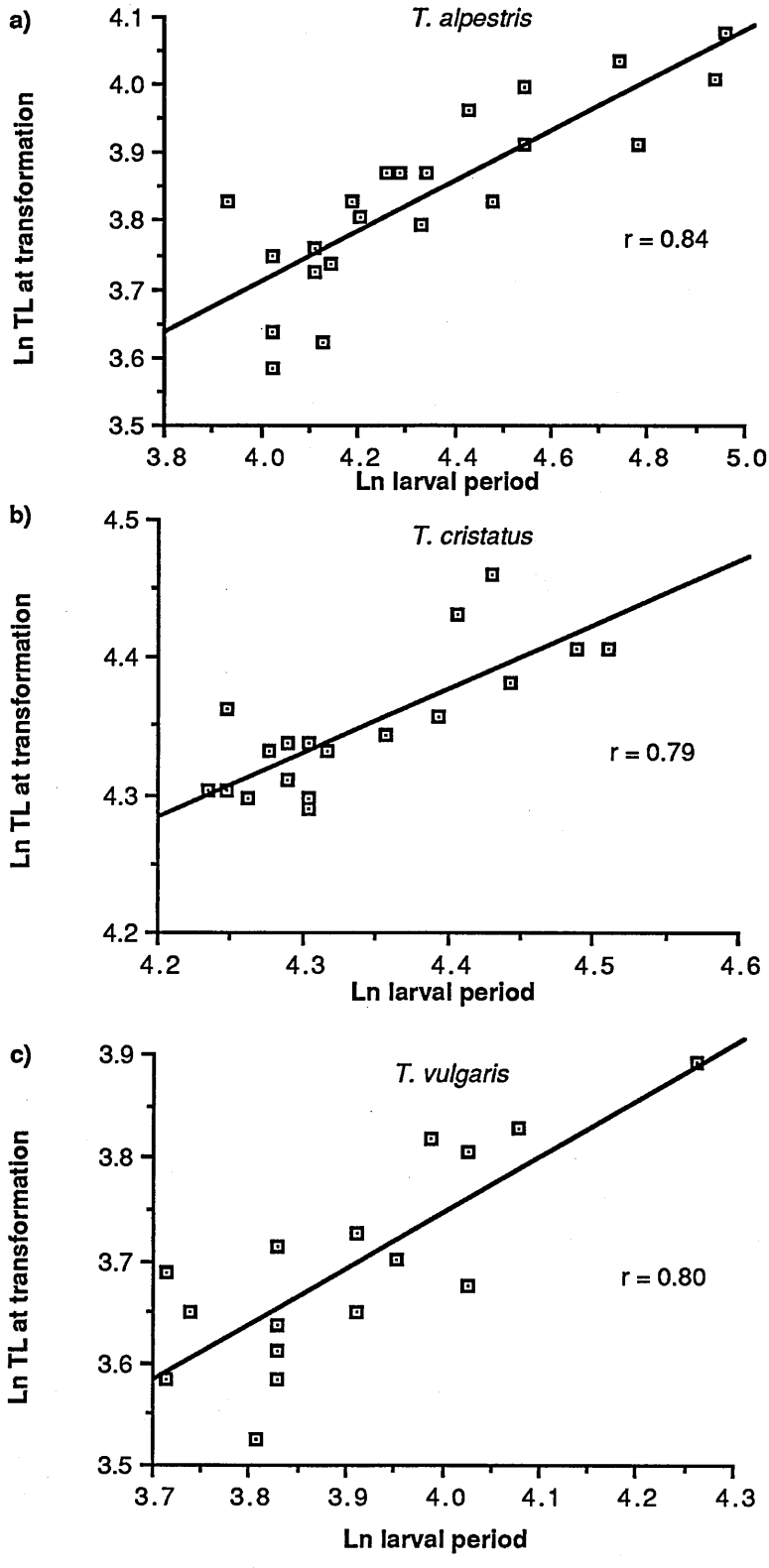


Fig. 4.2. Body size of individual metamorphs plotted against larval period. Body size is plotted as Ln TL (mm) and larval period is plotted as Ln length of larval period (in days). a) *T. alpestris*, b) *T. cristatus*, c) *T. vulgaris*.

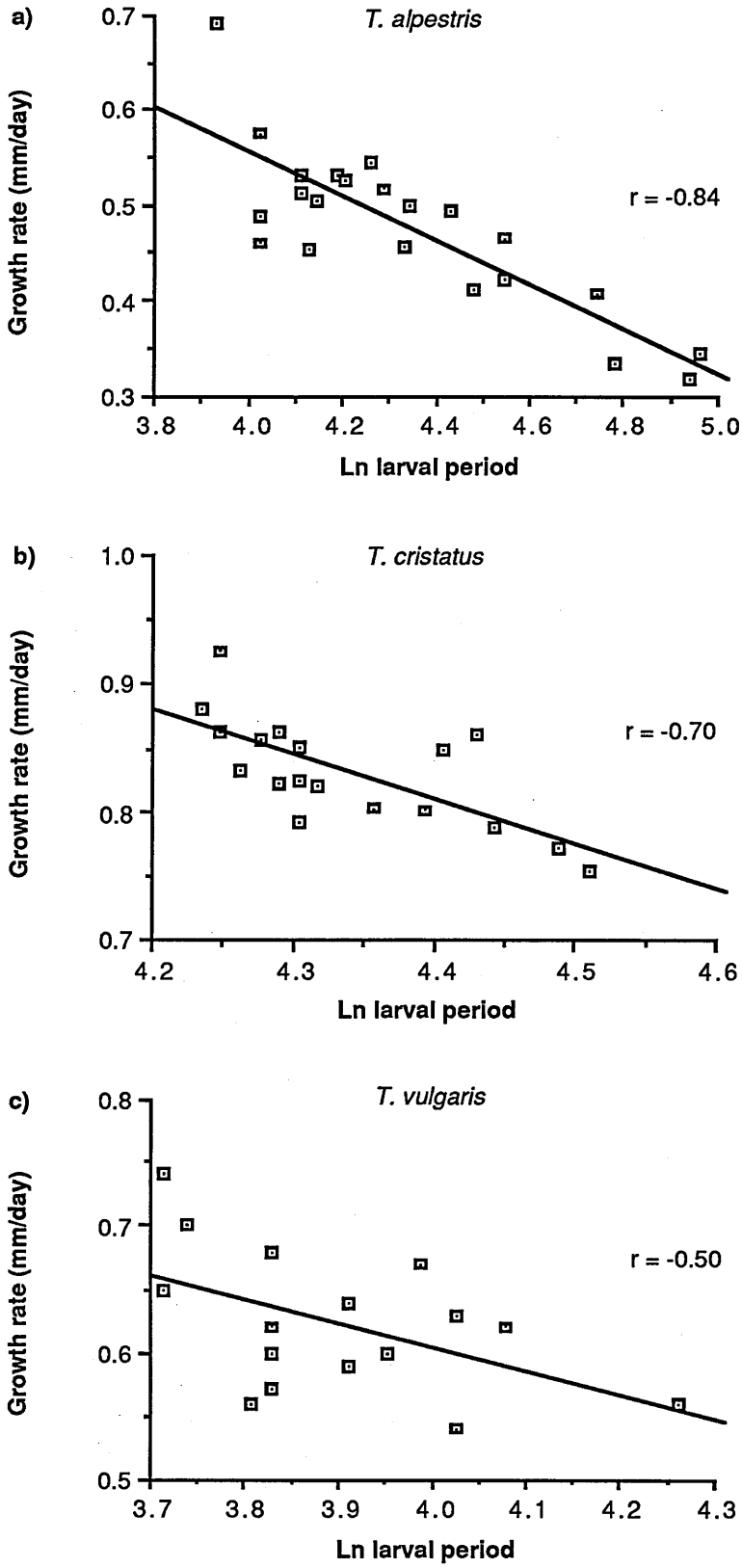


Fig. 4.3. Graphs to show larval growth rate plotted against larval period for individual larvae. Growth rate is expressed as increase in TL (mm) per day and larval period is expressed as log days.

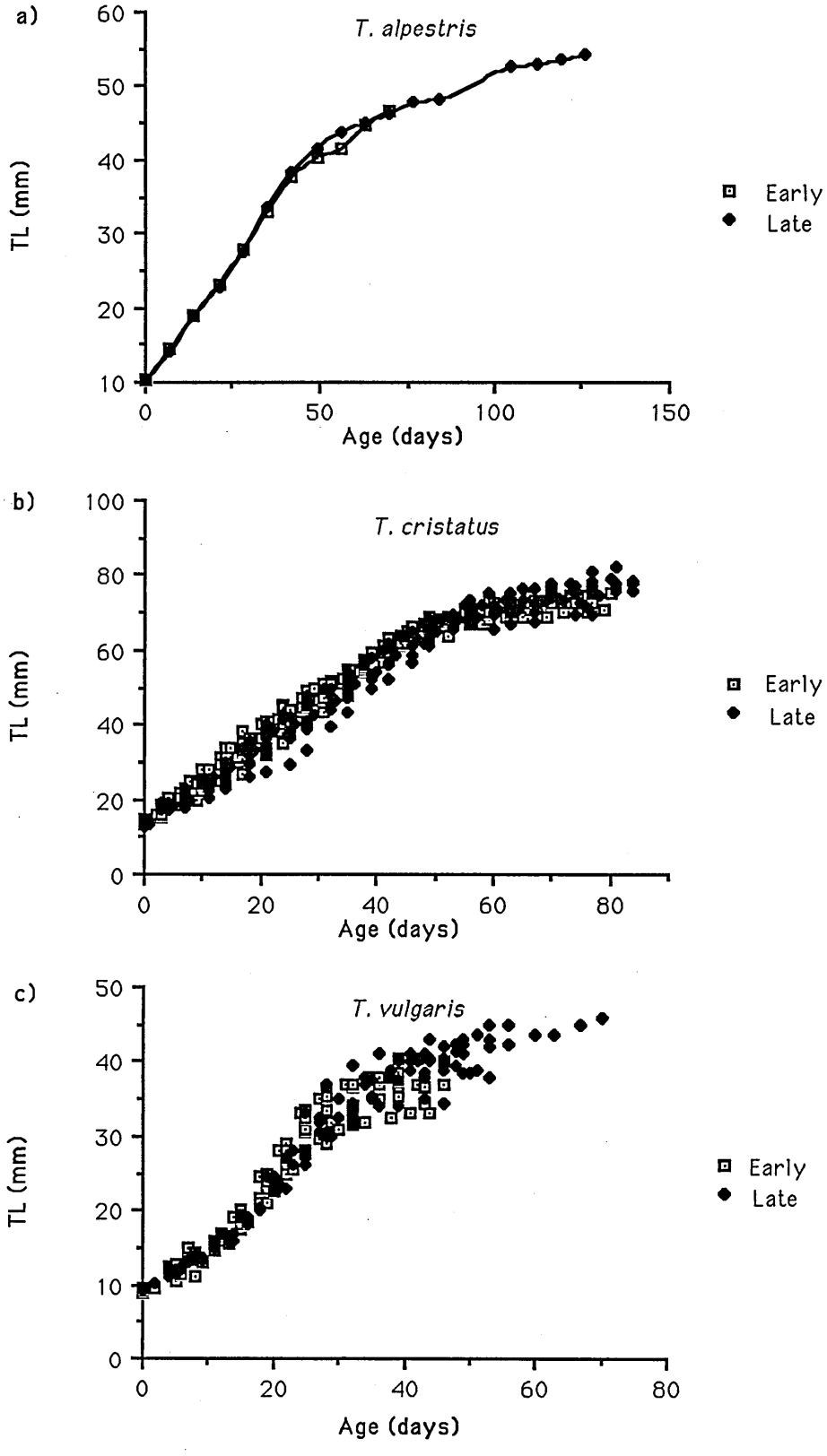


Fig. 4.4. Growth of early and late metamorphs. 'Early' metamorphs are the first 50% to transform and 'late' metamorphs are the later 50% to transform.

Discussion Newt larvae grown under similar conditions expressed a wide variety of growth and developmental responses. The uniformity of the rearing environments suggests that this variation is not environmentally induced and hence may be the result of genotypic variation or non-genetic maternal effects on larval growth rate. Maternal effects are examined in Section 5.4, with the conclusion that non-genetic maternal effects have little influence on the growth of *Triturus* larvae under laboratory conditions. Hence it can be concluded that the variation in larval growth patterns recorded in the present study is consistent with the hypothesis that there is much genetic variance in larval growth parameters. Evidence of genotypic variation in growth and developmental rates has been given, using *in vitro* fertilization techniques, for larval anurans. Travis (1981) showed that larval growth rate and larval period varied between sibships in *Pseudacris triseriata*; Newman (1988) found that *Scaphiopus couchii* sibships showed varying larval periods in laboratory and field enclosures; Mitchell (1990) found that parental identity affected metamorph body mass and larval period in the laboratory and in the field. Much of the variance in larval period and body size that is observed in *Triturus* populations in the field (eg. Verrell 1985a) could then be the result of variation intrinsic to the larvae themselves, rather than an expression of the differential effects of exploitative competition for food or behavioural interactions. To establish what other, if any, factors influence the growth of newt larvae, it is necessary to perform further experiments (Chapter 5).

Berven and Gill (1983) proposed that genotypic variation in the timing of transformation exists due to a lack of selection pressure to transform at any specific time. This approach suggests that the timing of transformation is of no ecological significance and is challenged by the view of Newman (1988), which states that although there is no single optimum time to transform, there are important consequences to the timing of this event. Newman proposed that genetic variance existed within a population as an evolutionary response to an unpredictable environment. His study species, *Scaphiopus couchii*, inhabits a particularly unpredictable environment, since this species breeds in

temporary ponds in a desert. Ponds dry rapidly, and pond duration is related to pond depth (which the adults do not seem to be able to use as a cue to pond permanence) and rainfall. *Triturus* species do not inhabit such an extreme environment, but they are nevertheless faced with breeding sites that vary in quality and permanence. Hence variability in *Triturus* larval ponds may parallel variability in *Scaphiopus couchii* habitat, and both may select for genetic variability in larval growth and developmental responses, since genotypic variability in these responses will provide higher parental fitness than genetic homogeneity. Of Newman's experimental ponds, those that dried rapidly allowed only the rapid developers to complete metamorphosis, at a small body size, whereas longer-lived ponds enabled all larvae to transform, such that the slower developers attained a larger body size than the fast developers. From the present data it is not possible to establish whether larval period varies within the progeny of a single female, or between different females.

The finding that larger metamorphs have longer larval periods, but slower growth rates than small metamorphs, shows that within the variation expressed in these two parameters, larvae do not show a trade-off between rapid growth and rapid development. Fast and slow growing larvae are equally likely to either prolong or shorten the larval period. Under the identical growth conditions provided in this study, all larvae appear to be growing on a similar growth trajectory, but develop at different rates, so that they leave the larval growth curve at different times (such a situation is represented diagramatically in Fig. 4.5). It is this variation in differentiation rate, imposed on a fairly rigid growth trajectory that generates most of the variation in body size recorded in the present study.

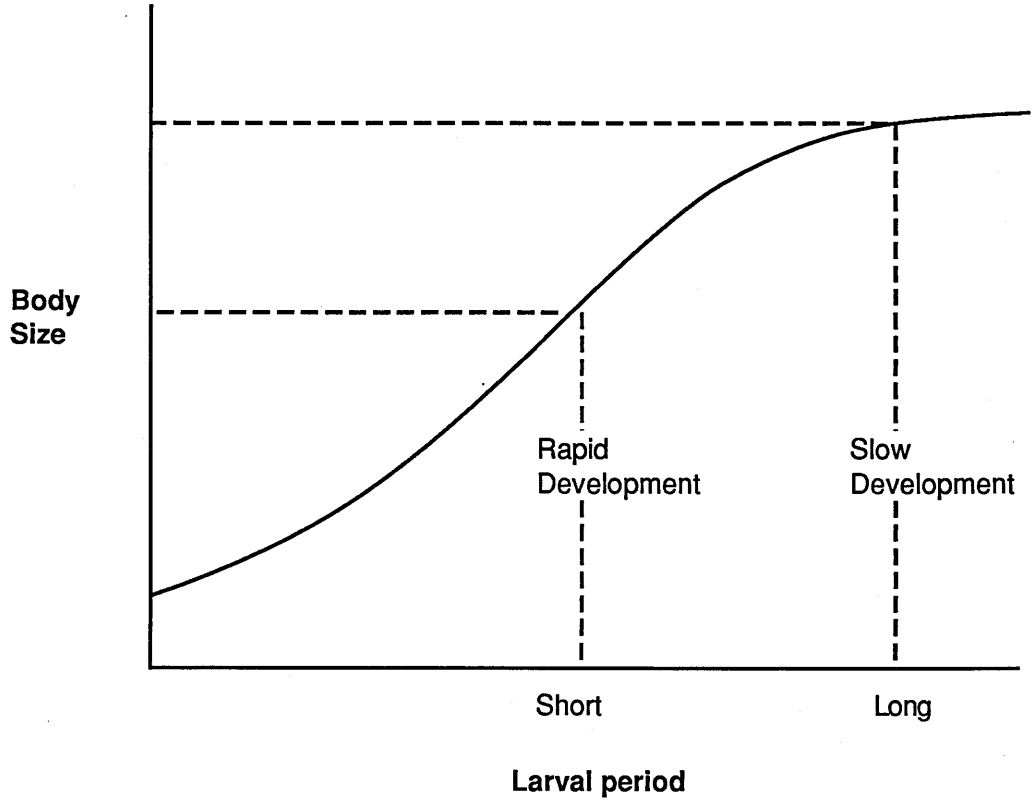


Fig. 4.5. Diagramatic representation of larval growth trajectory, for a constant set of growth conditions, to show how variation in rate of development affects larval period and body size at transformation.

It should also be noted that those individuals that prolonged larval period, thus gaining large body size, did so by extending the shallowest part of the growth curve, so that their overall larval growth was in fact slower than the earlier transforming larvae. In order to ascertain whether the gain in size is a real advantage, it is necessary to find out more about growth during the eft stage. Eft growth that is as rapid as growth during the latter stages of the larval growth curves shown in Fig. 4.1 will allow the earlier metamorphs to attain a similar body size as the later transforming individuals.

Increasing body size at transformation with larval period does not appear in reports of *Triturus vulgaris* (Harrison 1985, Verrell 1985a) or *Ambystoma talpoideum* (Semlitsch et al. 1988) transforming in natural ponds, and Sprules (1974) only recorded it in two out of twelve groups of *A. gracile* reared in a laboratory. However, Shoop (1974) records this effect in *A. maculatum* transforming in a natural pond. There are two possible reasons for the discrepancy between the results of the present study and those from field studies of newts (Harrison 1985, Verrell 1985a). The prolonged period of oviposition, or changing larval growth conditions could mask this effect in the natural situation. Travis (1983), developing the competitive release ideas of Savage (1952) and Collins (1979), claims that a size increase with larval period is only to be expected when larvae are reared in groups, since as more larvae transform and leave the group, growth conditions must improve for the remainder. How reliably this effect transfers to the field is unclear, since Savage (1952) observed it in *Rana temporaria* and *Bufo bufo*, but Smith (1987) notes that in natural ponds, body size of *Hyla gratiosa* decreases with larval period. Data from Travis's (1980) paper show that when *Hyla gratiosa* are reared as singletons, the relationship between body size and larval period does indeed disappear. Since *Triturus* larvae retain this relationship when reared as singletons, their growth appears to differ from that of hylids.

The present rearing study shows that there is much variation in the larval period of newts grown under similar conditions. In the field, urodele larval growth characteristically shows much within-population variation in length of the larval period and size at

completion of metamorphosis (eg. *Triturus vulgaris* [Verrell 1985(a)], *A. maculatum* [Shoop 1974]). However, this variation becomes even more pronounced when the phenomena of overwintering and paedomorphosis are considered. Overwintering describes the prolongation of the larval period by those individuals that do not transform within the usual first summer or autumn seasons of life, but instead remain in the larval form during the course of the winter and transform the next year (eg. Verrell 1985(a), *T. vulgaris*). Paedomorphosis is used to describe the state of attainment of sexual maturity whilst still retaining juvenile morphology ('displacement of ancestral features to later stages of the ontogeny of descendents' Gould [1977]) and is a relatively widespread phenomenon among urodeles. Paedomorphosis can be obligatory, as in certain species from the genera *Siren*, *Cryptobranchus*, *Amphiuma*, *Necturus* and *Proteus*, or facultative, as in *Ambystoma talpoideum*, *A. tigrinum*, *A. gracile* and *Notophthalmus viridescens*, (Semlitsch and Wilbur 1989). Paedomorphosis is reported to occur in all three British species of *Triturus* in which this state is sometimes referred to as neoteny (Frazer 1989 p. 118).

Explanations of overwintering and paedomorphosis differ. Overwintering is given a causal explanation, since it is proposed that poor larval growth in some circumstances does not enable a larva to transform within the typical time period. Paedomorphosis is explained adaptively as an omission of the terrestrial stage of the life cycle that occurs as a response to favourability of aquatic conditions compared to the terrestrial environment (eg. permanent, predator-free water surrounded by a harsh terrestrial terrain [Gould 1977]) or as a response to favourable recent growth history (Harris 1989). There is some evidence from studies of facultatively paedomorphic populations that supports this theory. Morin et al. (1983) noted that low larval densities tended to be associated with a greater expression of paedomorphosis in *Notophthalmus viridescens* and pond drying experiments (eg. Semlitsch and Gibbons 1985 and Semlitsch 1987) show that ponds that do not dry tend to produce a greater proportion of paedomorphs in *A. talpoideum*. Note, however, that there are also confounding effects of temperature, density and food that occur as ponds dry

which are recognised by Semlitsch and Gibbons (1985).

The variance in larval period noted in the present study can be seen as part of the process that produces overwintering in wild populations. A cohort of larvae will complete metamorphosis over a wide range of time, and it is conceivable that under certain conditions that prolong larval period (eg. low temperature or food supply, examined in Chapter 5) the later larvae to transform may suffer such a lengthened larval period that transformation occurs in the following spring or summer.

However, there was no evidence of paedomorphosis in any of the larvae reared during the course of this project. Paedomorphosis seems to have a genetic basis. *Ambystoma mexicanum* seems to have a recessive homozygous gene for paedomorphosis, as revealed by crosses with *A. tigrinum* (Tompkins 1978) and Semlitsch and Wilbur (1989) were able to alter the frequency of occurrence of paedomorphosis in *A. talpoideum* by selective breeding (only breeding from paedomorphic individuals) over five years. So, why does paedomorphosis occur in some wild *Triturus* populations but was never seen during the course of this study? Firstly it must be appreciated that this state is unusual in Great Britain. Paedomorphic *Triturus* populations are more typically found in continental Europe, in mountainous regions, where hostile terrestrial environments may be the factors selecting the evolution of paedomorphosis (eg. Kalezic and Dzukic 1986).

Paedomorphs in Britain seem to occur in two forms, which are described by Smith (1950). The first is an albinistic form, yellow or cream with pigmented eyes and red gills. The second form is a similar colour to transformed adults, looking much like a metamorphic adult with gills. Smith notes that the former type is smaller than normal adults, whilst the latter is bigger. Frazer (1989) raises the question of whether the former type can reproduce, especially since lack of pigmentation may be linked to a pituitary fault and the pituitary also controls development. If such animals cannot reproduce, then by definition they cannot be regarded as being paedomorphs and they may well represent the result of aberrant development rather than an adaptive response to prolong aquatic life. The second type of paedomorph reported seems to be similar to paedomorphs from continental

Europe, although Kalezic and Dzukic (1986) recorded that their non-albinistic paedomorphs were smaller than normal adults.

The lack of any paedomorphic responses in the present study could be due to three possibilities. Either lowland populations of *Triturus* species do not contain the genetic coding for paedomorphosis (the occasional cases that are reported must be regarded as either not being true paedomorphs, or the result of mutation), or recessive genes for paedomorphosis may be present, but at low frequencies due to strong selection to be able to transform at a relatively early age in order to leave deteriorating ponds and colonize new ones. Thirdly, the genetic capacity for paedomorphosis is present but the correct environmental conditions needed for its expression were not provided in these laboratory studies. The differing rate of expression of paedomorphosis under varying larval growth conditions is evidence that paedomorphosis may be an epistatic response (eg. Sprules 1974, Morin et al. 1983, Semlitsch and Gibbons 1985, Semlitsch 1987).

As an alternative to simple genetics, perhaps there is an additive genetic effect for differential rate of development of certain features which leads to the retention of some larval features, namely gills and fins. In the present study the larvae with long larval periods, during the period of protracted larval life, looked like efts with gills rather than transforming larvae. Thus the samples of larvae as a whole appeared to contain a mixture of some individuals that underwent rapid differentiation of all features so that they transformed after a short larval period, and some larvae that seemed to have a relatively slower rate of differentiation of gills and tail fins relative to other characters. Harris's (1989) morphometric study of *Notophthalmus viridescens* finds that paedomorphs tend to be more similar to metamorphic individuals than they are to larvae. Personal observation of paedomorphic smooth newts from Yugoslavia (captured by C. Raxworthy) reveals that these animals also look like adults with gills, rather than larvae with gonads.

Conclusion Samples of larvae from three newt species (*T. alpestris*, *T. cristatus*, *T. vulgaris*) grown under similar laboratory conditions showed much intrinsic variation in larval period and size at transformation. For all three species of larvae, body size at

transformation increased with larval period, but overall growth rate decreased with increasing larval period. The Wilbur-Collins (1973) model of metamorphosis does not explain this variance in larval period, since growth trajectories of larvae with long and short larval periods are similar. Most of the variation in size of metamorphs is due to differential rate of development of larvae that are growing on a similar growth curve.

Chapter 5
Factors Affecting Larval Growth

This chapter examines the effect of four factors that are presumed to affect larval growth. These are:

1. The temperature at which larvae are reared.
2. The availability of prey to developing larvae.
3. The density of larvae.
4. Maternal effects.

Larvae were grown in the laboratory, as described in Chapter 4, and were of three species: *Triturus cristatus*, *Triturus vittatus* and *Triturus vulgaris*.

5.1 Effect of Temperature on Larval Growth

Introduction Temperature effects on the growth of amphibian larvae and fish have been well-documented. In fish, growth rate increases with temperature to a maximum and then declines. This is presumably because food intake does not increase as fast as metabolic rate. The latter increases exponentially with temperature (Weatherley and Gill 1987). Among anuran larvae, higher temperatures tend to increase growth rates and decrease larval period, but result in a smaller body size at transformation. Smith-Gill and Berven (1979) have evidence of this effect in a series of laboratory studies. In introducing their model of metamorphosis, they presented the results of rearing *Rana pipiens* larvae at different temperatures, with the result that at cooler temperatures, although growing more slowly, larvae were larger at all developmental stages, including transformation, than larvae reared at warmer temperatures. Berven et al. (1979) recorded a similar effect of temperature on the growth of larval *Rana clamitans* in the laboratory and in the field. Similar results were

obtained by Berven (1982) for *Rana sylvatica* and by Harkey and Semlitsch (1988) for *Pseudacris ornata*, both grown in the laboratory. Lower temperatures prolonged larval period and increased body size at transformation.

The greater body size observed at lower temperatures is difficult to explain using the Wilbur-Collins (1973) model of metamorphosis because lower temperatures result in slower larval growth rates. A larva monitoring its own growth rate should transform at a smaller body size and so escape its poor larval growth environment. However, all of the above studies show the opposite effect. The Smith-Gill and Berven (1979) model of metamorphosis seeks to explain this decrease in size of metamorphs, as temperature increases. It postulates that the processes of growth and development may be differentially affected by temperature. Hence high temperatures cause a greater increase in rate of development than in rate of growth, so that although individuals grow faster, they develop faster still and thus transform at a smaller size. It is possible that the mechanism of this effect may be parallel to the effect of temperature on fish growth. Higher temperatures increase metabolic rate, so that although development proceeds more rapidly, the raised cost of maintenance leaves less of the energy budget for growth, with the result that larvae are actually smaller at all developmental stages.

Developmental studies of urodeles show that differentiation rate increases with temperature eg. *Ambystoma tigrinum* (Shrode 1972), *Triturus carnifex* (Horner and MacGregor 1985). There is less quantitative information concerning growth, and the conclusions seem to lack unanimity. As in the developmental studies, Uhlenhuth (1919) recorded that low temperatures slowed differentiation rate, but also tended to lead to larger body sizes at transformation, in laboratory studies of *A. opacum*. In another laboratory study, Sprules (1974) found that low temperatures prolonged larval periods in *A. gracile*, but could not detect any consistent effect on body size. Bizer (1978), in a field study of *Ambystoma tigrinum* larvae, found that pond temperatures affected growth rate. Higher pond temperatures tended to increase larval growth rates, but produced smaller metamorphs. Petranka (1984) recorded the same effect in the laboratory, but found that in

the field, warm ponds yielded bigger metamorphs than small ponds. Harris (1984) in transplant experiments using *Ambystoma* larvae, concluded that food had a greater effect on metamorph size than temperature, but noted that larvae in colder (montane) ponds had longer larval periods than warmer (lowland) ponds. Results of field observations should always be treated with caution, since an effect such as temperature variation between ponds may also be accompanied by other, confounding, variables. For example warmer ponds may be more productive, in terms of prey densities, or they may have lower levels of dissolved oxygen, both of which may also affect larval growth responses. Thus Petranka's (1984) field observation that contradicts his laboratory findings, that lower temperatures produce larger metamorphs, may be due to such confounding variables.

To conclude, among amphibian larvae, it seems that temperature has a predictable effect on rate of growth and development: low temperatures retard differentiation, prolonging the larval period and reducing rate of growth, but producing larger individuals at transformation. The present study examined growth of larvae of *Triturus vulgaris* reared individually at different temperatures in the laboratory.

Methods Eggs and larvae were treated as described in Section 4.2. Each larva was maintained in an individual beaker. Larvae were reared under four constant temperature regimes: 12°C (1987), 20°C, 25°C and 15°C (1988). The choice of temperatures was arbitrary since there is little data available concerning natural pond temperatures. However, temperatures recorded at a local pond suggest that newt larvae may experience a range of temperatures between 5°C and 25°C. Temperature was maintained by placing the beakers and trays in constant temperature cabinets, except in the case of the 20°C larvae which were kept at a constant temperature by placing the individual beakers into three shallow, heated water baths. The water baths heated consisted of three 60 x 45 x 25 cm aquaria heated by an aquarists' heater-stat. The baths were placed in a cooled room so that ambient temperature stayed at 12°C. Each bath had a glass lid to reduce heat and water loss. The beakers were rotated between baths and within the baths twice a week, to minimize any possible positional effects. All larvae were maintained under a 16:8 L:D photoperiod.

The larvae at 15°C were grown only to stage 53, since at this temperature larval periods are so long that the experiment would run for too long.

I attempted to rear 16 larvae to transformation at each temperature (or to stage 53 in the 15°C group). Details of numbers used in this study are given in Table 5.1.

Temperature °C	No. started	No. died	No. discarded (malformed)	No. transformed
25	22	6	3	13
20	28	11	1	16
15	16	1	1	-
12	19	6	0	13

Table 5.1. Numbers of *T. vulgaris* larvae reared and mortality at each temperature.

Larvae were measured three times a week at 25°C, twice a week for 20°C and once a week at 15 and 12°C, although in the final analysis only size at and timings to three stages were used: Gallien and Bidaud stage 40 (day zero of larval period), stage 53 and stage 55c (transformation). Larval period was calculated as time in days between stage 40 and transformation. Larval growth rate was calculated as the increase in TL per day:

$$\text{Larval growth rate} = \frac{TL_{(i+1)} - TL_{(i)}}{t\,(i+1) - t\,(i)}$$

$TL_{(i)}$ = TL at start of growth interval

$TL_{(i+1)}$ = TL at at end of growth interval

t (i) = time at start of growth interval

t (i+1) = time at end of growth interval

Size at, and growth rate to, stage 53 were used as measures of stage specific growth, and size during the larval period, in case this differed from mean larval growth. This stage was selected since detectability of the fifth digit on the hind limb is a discrete event in time; it is easily observed and occurs approximately half-way through the larval period. Size at transformation is given as TL and SVL. TL was recorded to allow comparison with larval body size, and SVL to allow comparison with other studies, since SVL is a commonly used measure of urodele body size.

Results Size at and time taken to attain stage 53 are presented in Table 5.2.

Size (TL) at stage 53 The data do not display homogeneity of variances $F_{max} = 4.854$ (> 2.50 at 5% level) so the Kruskal-Wallis test was used: $H = 40.039$, $p < 0.001$. There is a significant effect of temperature on TL at this stage. TL increases with temperature up to a certain temperature, around 20°C and then above this point TL decreases again.

Time taken to attain stage 53 The Kruskal-Wallis test was used because of non-homogeneity of variances, $F_{max} = 154.15$ (> 2.50 at 5% level). $H = 57.988$, $p < 0.001$. There is a significant effect of temperature on rate of development to this stage. Higher temperatures increased rate of development.

Growth rate to stage 53 The data do not display homogeneity of variances $F_{max} = 30.99$ (> 2.5 at 5% level) so the Kruskal-Wallis test was used: $H = 52.981$, $p < 0.001$. There is a significant effect of temperature on growth rate to stage 53. Higher temperatures produce faster growth rates.

Size at and time taken to attain transformation are presented in Table 5.3.

Size (TL) at Transformation Variances were found to be equal, $F_{max} = 1.80$ (< 2.687 at 5% level) so these data were analysed using a one-way ANOVA. F-ratio = 5.799, $p < 0.006$. There is a significant effect of temperature on TL at transformation. TL increases with temperature up to 20°C and declines above this temperature.

Size (SVL) at Transformation Variances were found to be equal, $F_{max} = 1.87$ (< 2.687 at 5% level) so these data were also analysed using a one-way ANOVA. F-ratio = 3.845, $p < 0.030$. There is a significant effect of temperature on SVL at transformation, similar to that on TL.

Time taken to attain transformation (Larval period) Variances were not found to be equal, $F_{max} = 243.0$ (> 2.687 at 5% level) so these data were analysed using the Kruskal-Wallis test. $H = 32.903$, $p < 0.001$. There is a significant effect of temperature on larval period. Higher temperatures decrease larval period.

Growth rate to transformation Variances were not found to be equal, $F_{max} = 20$ (> 2.687

at 5% level) so these data were analysed using the Kruskal-Wallis test. $H = 29.170$, $p < 0.001$. There is a significant effect of temperature on specific growth rate to transformation. Higher temperatures produce faster growth rates.

TL (mm) at stage 53

Temp.	mean	s.d.	CV	var	n
25	21.5	2.057	9.57	4.231	14
20	26.2	1.491	5.70	2.224	16
15	25.1	0.963	3.84	0.927	14
12	21.0	2.121	10.10	4.500	19

Time (days) taken to attain stage 53

Temp.	mean	s.d.	CV	var	n
25	15.8	1.251	7.93	1.566	14
20	22.1	1.692	7.67	2.862	16
15	38.7	3.496	9.03	12.220	14
12	120.9	15.53	12.85	241.4	19

Growth rate (mm per day) to stage 53

Temp.	mean	s.d.	CV	var	n
25	0.782	0.124	15.87	0.015	14
20	0.763	0.060	7.89	0.004	16
15	0.407	0.044	10.83	0.002	14
12	0.098	0.022	22.88	4.8×10^{-4}	19

Table 5.2. To show effect of temperature on growth of *T.vulgaris* larvae to, and size at, Gallien and Bidaud stage 53. Data for four different rearing temperatures (25, 20, 15 and 12°C) are given as means with standard deviations (s.d.), coefficients of variation (CV), variances (var) and sample sizes (n).

TL (mm) at transformation

Temp.	mean	s.d.	CV	var	n
25	36.5	4.366	11.97	19.061	13
20	40.37	4.157	10.30	17.283	16
12	35.8	3.251	9.09	10.567	13

SVL (mm) at transformation

Temp.	mean	s.d.	CV	var	n
25	19.2	2.164	11.30	4.683	13
20	20.7	1.825	8.81	3.332	16
12	19.0	1.581	8.32	2.500	13

Time (days) taken to attain transformation

Temp.	mean	s.d.	CV	var	n
25	39.6	4.788	12.09	22.923	13
20	50.1	7.870	15.72	61.929	16
12	275.4	74.638	27.10	5570.8	13

Growth rate (mm per day) to transformation

Temp.	mean	s.d.	CV	var	n
25	0.689	0.092	13.33	0.008	13
20	0.625	0.055	8.88	0.003	16
12	0.100	0.020	20.10	4×10^{-4}	13

Table 5.3 To show effect of temperature on growth of *T.vulgaris* larvae to, and size at, transformation. Data for three different rearing temperatures (25, 20 and 12^{o}C) are given as means with standard deviations (s.d.), coefficients of variation (CV), variances (var) and sample sizes (n).

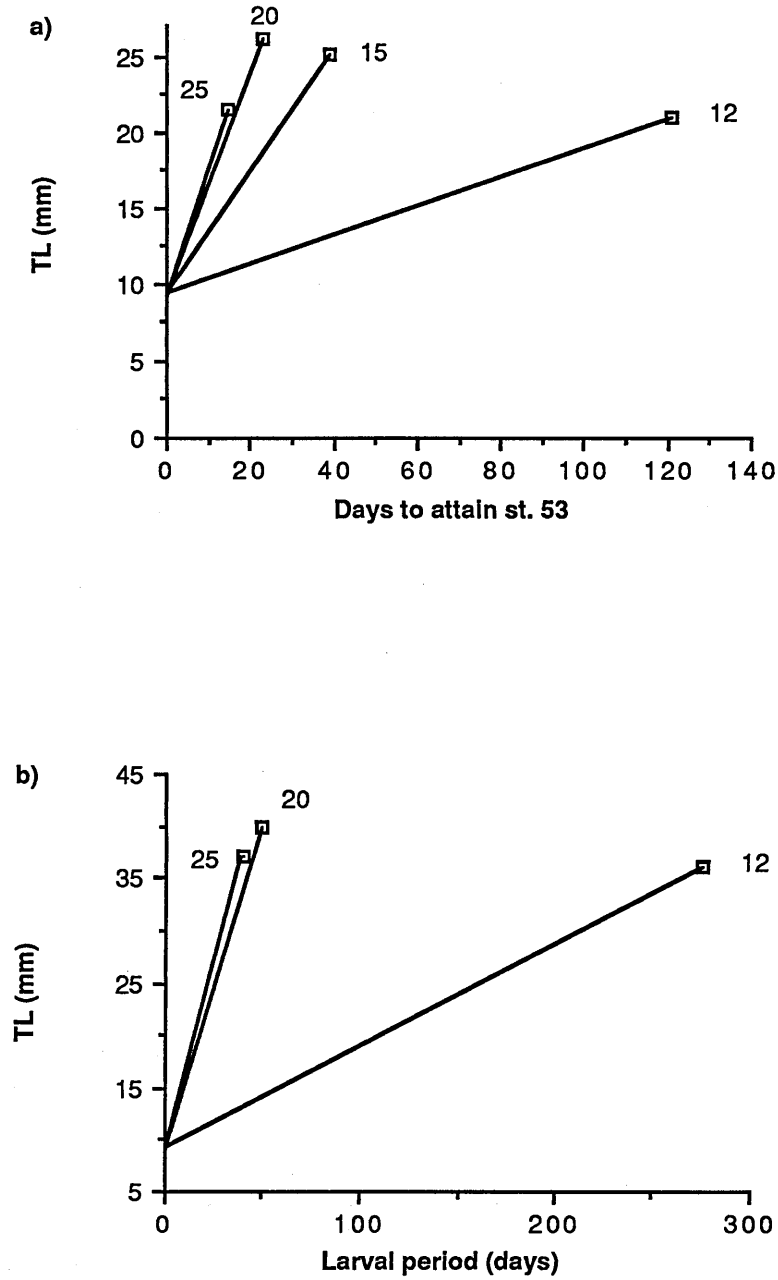


Fig. 5.1. Graphs to show effect of temperature on growth of *T. vulgaris* larvae. (a) Shows overall growth rate to Gallien and Bidaud stage 53 at 25°C, 20°C, 15°C and 12°C. (b) Shows overall growth rate to transformation at 25°C, 20°C and 12°C.

Discussion This study demonstrates that *Triturus vulgaris* larvae are sensitive to temperature with respect to growth and development responses. The temperature driven responses recorded in this study show that smooth newt larvae do not behave in a manner predicted by the Smith-Gill and Berven model (1979). If the two processes of growth rate and differentiation were 'uncoupled', as the model suggests, so that differentiation increases rapidly with temperature, whereas growth rate increases more slowly, then a consistent decrease in body size at transformation would be expected as temperature increased. In the present study, metamorph body size increased up to 20°C and then decreased at 25°C.

Superficially, growth of larval smooth newts appears to behave differently to that of fish, since there is no apparent maximum growth rate. However, this difference is probably artificial, since there may well be a maximum growth rate at temperatures above those used in the present study. However, the ecological significance of growth at temperatures higher than 25°C must be doubtful in a temperate species. If this maximum growth rate does exist, then it is at a higher temperature than the temperature that achieves maximum body size (approximately 20°C). A difference between the temperatures that maximize body size and growth rate may in fact be due to a similar process to that which limits fish growth. As temperatures increase, then rate of physiological processes, such as development and metabolic maintenance, will also increase, but the rate of energy assimilation does not increase as rapidly, so that there is less energy available for somatic growth at any developmental stage, at temperatures above approximately 20°C. There is evidence to suggest that urodele physiology may behave in this manner because *Notophthalmus viridescens* is more efficient at converting food to weight increase at low temperatures than high temperatures (Springer 1909).

It is not possible to tell, from Weatherley and Gill (1987), how temperature affects body size at comparative developmental stages in fish, but it would be interesting to know if fish growing at higher rates due to warmer temperatures always develop faster than those at cool temperatures, like the newt larvae of the present study, and irrespective of the effect

of temperature on energy assimilation. Alternatively, the developmental rate of fish may always be dictated by body size as suggested by Weatherley and Gill's assertion that sexual maturity is size dependent in fish.

T. vulgaris is eurytopic in its choice of breeding ponds. The flexibility of larval period as a response to temperature may be an adaptive response to enable larvae to cope with variable larval growth conditions. For example, small, warm ponds are most prone to desiccation, and this temperature regime will minimize larval period whereas bigger, and hence cooler, ponds will be less likely to dry out, so that the more slowly developing larvae will be able to grow to transformation with less risk of pond desiccation before they have completed development. Whether this response is an adaptive response to avoid mortality due to pond desiccation or whether it is merely a reflection of the general principle that most physiological processes are faster at warmer temperatures (Schmidt-Nielsen 1979), is impossible to say. Wilbur (1987) found that toads (*Bufo americanus*) seemed to be able to respond to lowering of water levels, in artificial pond experiments, by transforming earlier than in deep water ponds, and Newman (1989) found that *Scaphiopus couchii* were able to transform earlier in natural, drying ponds. Newman points out that this effect is potentially confounded by changes in the temperature regime (shallow ponds are warmer) and changes in water chemistry as ponds dry out. However, Crump (1989a), removing these effects in a laboratory 'pond drying' experiment showed that *Hyla pseudopuma* was still able to reduce larval period in response to decreasing water levels, at the cost of reduced body size at transformation.

It is evident that larval period is dramatically affected by temperature, and that warmer temperatures, up to about 20°C, produce bigger metamorphs. Timing of and size at transformation may be related to fitness (see Chapter 4.1), so warmer temperatures would be beneficial to individual larvae, and there may be an optimum temperature under conditions where growth to a large body size is of greater survival value than is rapid development. This optimum is around 20°C - a temperature likely to be only infrequently encountered in the breeding ponds of *T. vulgaris* in this northern part of its distribution.

Thus, for a larva it may be beneficial to select warmer areas of the pond in order to increase body size and speed of development. Whether *Triturus* larvae behaviourally thermoregulate is not known, but Harrison (1985) did find that newt larvae (*Triturus helveticus* and *T. vulgaris*) tended to remain close to the surface of his study pond, which may be a thermophilic response. Studies of larvae of *Ambystoma tigrinum* (Lucas and Reynolds 1967, Heath 1975), *A. texanum*, *A. maculatum* (Keen and Schroder 1975) and the paedomorphic *Cryptobranchus alleganiensis* and *Necturus maculosus* (Hutchinson and Hill 1976) show that these species demonstrate some sort of behavioural thermoregulation in laboratory controlled thermal gradients. Temperature selection does not seem to be very precise in *Ambystoma* species; they seem to avoid extremes rather than select an optimum. *A. tigrinum* remained between 14 and 33°C (Lucas and Reynolds 1967), *A. texanum* and *A. maculatum* kept between 13 and 29°C. These ambystomatid experiments used ten (or four in the case of Heath 1975) larvae in the same gradient at one time. Studies of single larvae may yield more precise thermoregulatory behaviour since individuals will not be disturbed by other larvae and results will not be distorted by avoidance behaviour.

In the field, Heath (1975) has recorded the temperatures of ponds in the areas that *Ambystoma tigrinum* aggregated and found that these tended to be the warmer areas of the ponds (13-25°C). There has been no thermal gradient work on *Triturus* larvae, although there are data on terrestrial forms. Strübing (1954) examined temperature preferences of adults and juveniles in a thermal gradient. His newts selected quite surprisingly high temperatures (see Table 5.4). His juveniles selected higher temperatures than adults, possibly to maximize growth rates. Their selected temperatures are similar to the optimum estimated for maximizing body size by the larvae of *T. vulgaris* in the present study. However Özeti (1964) recorded a lower preferred temperature of 18.4°C for *T. vulgaris vulgaris* from Western Anatolia.

	Adult	Juvenile
T. vulgaris	23.5	24.6
T. cristatus	20.6	24.7

Table 5.4 To show mean temperatures (^{o}C) selected by *T. vulgaris* and *T. cristatus* in the terrestrial phase (from Strübing 1954).

5.2 Effect of Differing Food Levels on Larval Growth

Introduction Little is known of how food levels affect natural populations of newt larvae. Before experimental manipulation of prey levels, it is important to consider whether larvae are ever food limited in the natural situation. Ponds can be very productive environments and so it is quite possible that larval populations are regulated by density independent factors before prey ever becomes limiting, as proposed by the Andrewartha and Birch (1954) theory of population regulation (for a further discussion of density effects see Section 5.3). However, Harrison (1985) noted that 13% of newt larvae (*T. helveticus* and *T. vulgaris*) had empty stomachs, which suggests that prey may be limited in some ponds, at some times. Smith and Petranka (1987) have evidence of two mechanisms through which prey may become a factor limiting larval growth. In ponds with low densities of small prey items (zooplankton) large larval *Ambystoma jeffersoniam* seemed to suffer low food acquisition rates relative to body size. They also noted that in ponds where prey composition consisted of large species, urodele larvae may suffer reduced food intake due to gape-limitation. An individual larva may not be able to capture prey items over a certain size due to the limits imposed by the size of the gape. Under such conditions larvae may suffer reduced growth rate, and faster growing larvae would benefit from being able to acquire more prey (relative to body size) through growing larger. If such mechanisms operate on *Triturus* larvae then food-limitation may affect growth in some situations.

The effects of differing levels of food on amphibian growth may seem obvious - lower food levels will produce smaller larvae. However the effect of low food levels on the timing of transformation and on the size at transformation produces three alternative predictions:

1. The larval period stays the same - but metamorphs are smaller.
2. Longer larval period - metamorph size stays the same.

3. Longer larval period - metamorphs are smaller.

Theoretically, a further alternative is also possible. Adopting an argument similar to the Wilbur-Collins (1973) model of metamorphosis, in that larvae alter the time spent in the larval habitat in order to maximize growth rate, it may be predicted that larvae should reduce their larval period in order to escape a poor growth environment. However, this is unlikely to be observed in practice, since the lack of prey items will limit the energy available for rapid developmental changes. Crump (1989b) provides an exception to this rule, since *Bufo periglenes* produces very large, yolk-rich eggs which allow the larvae of this species to complete transformation with no external food resources, and also to reduce the larval period in low food conditions. Hence, when the larval food source is in short supply, this species can transform earlier than when food is available. However, other amphibian larvae fall into the third category. They have longer larval periods and are still smaller than well-fed larvae at transformation eg. *Ambystoma texanum* (Petranka 1984), *Taricha torosa* (Kaplan 1985) and *Bufo woodhousei* (Mitchell 1990).

The present study examined the effect of two different levels of prey availability on the larvae of *Triturus vittatus* and *T. vulgaris*.

Methods

Triturus vittatus:

Collection of ova Eighteen female *Triturus vittatus* were captured (by C. J. Raxworthy) at Adapazari, Northwest Turkey. Oviposition occurred in transit and continued after the animals were installed in three aquaria similar to those described in General Methods. Ova were collected by two techniques. Some eggs were unwrapped from the weed substrate and placed in tissue culture dishes, one egg to a cell. The tissue culture dishes were placed in a laboratory, in a north facing window. Laboratory temperature fluctuated between 20 and 25°C. On hatching the larvae were transferred to rearing containers (28 x 24 x 15 cm containing 4 litres of water) via a 3mm bore pipette. The second technique was simply to transfer egg-laden weed to a container (28 x 24 x 15 cm) containing 2 litres of tap water. Such containers were kept with the tissue culture dishes above. Newly hatched larvae were collected daily and transferred to rearing containers. The former technique had the advantage that eggs in the individual cells can be labelled and identified. This treatment of the ova does not seem detrimental, and very high hatch rates were recorded : 269/275 = 97.8 %.

Rearing larvae Ten groups of 25 larvae were reared in plastic containers containing 4 litres of water, one group per container. Larvae were fed small *Daphnia*, obtained by netting a pond in a nearby park, and sieving the *Daphnia* through a small hand net. After three weeks *Tubifex* was added to the diet. At four weeks the larvae were removed, measured and staged. Some mortality had occurred so larvae were removed (chosen at random) so that the total number of larvae per culture was 20.

The cultures were then assigned, on a random basis, to one of two feeding regimes; eight cultures to 'high' and two cultures to 'low'. The high food regime larvae were fed *ad libitum* but the low food cultures were allowed to finish all food, then left for 24 hours before more prey items were added. As each larva emerged at transformation it was anaesthetized and measured as in Section 4.2.

Triturus vulgaris

Collection of ova As General Methods, Section 4.2.

Larvae were reared in transparent plastic containers, measuring 26 x 14 cm, containing 2 litres of water, placed in a laboratory as described for *T. vittatus*. Twenty larvae were reared in each container. Food was obtained as under general methods, except that three experimental feeding regimes were employed:

High - *ad libitum* feeding.

Low - food only provided on alternate days.

Reduced - switched from high to low feeding regimes after 35 days.

Two cultures were reared at each condition. At transformation all metamorphs were anaesthetized and measured as in General Methods.

Results

T. vittatus The effect of lowering the rate of feeding of the two low food cultures was to prolong the larval period and to decrease the size of emergent metamorphs (see Fig 5.2). T-tests were performed on the pooled data obtained from individual metamorphs from the low and high food levels. Results of these tests are given in Table 5.5.

Within the high food cultures it is apparent that body size tends to increase with larval period within each culture (for example see Fig. 5.2 c, d and e). The gradient of increasing TL with larval period is significantly different from a gradient of zero in six of the eight high food cultures (see Table 5.6). This effect is the same as that observed for single larvae in the previous chapter. However, this effect may not be as strong in the low food cultures, since one of these showed a negative gradient. The gradients of the regression line of log TL on log larval period, for all ten cultures, are given in Table 5.6.

Food Level		High	Low	
TL (mm)	mean	31.34	26.2	
	s.d.	2.90	3.11	t = 9.72, p < 0.001
	n	150	39	
Larval period (days)	mean	58.3	75.5	
	s.d.	8.35	18.30	t = 8.65, p < 0.001
	n	150	39	

Table 5.5 Mean body size (TL) at transformation and larval period (days) for high and low feeding regimes for *T. vittatus*. T-tests show significant differences between body size and larval periods of the two rearing regimes.

High food				Low food			
Gradient	t	p	n	Gradient	r	p	n
0.33	2.41	0.03 *	19	-0.02	0.21	0.83	20
0.47	3.15	<0.01 **	19	0.22	3.03	<0.01 **	19
0.49	5.19	<0.01 **	20				
0.30	1.58	0.14	17				
0.32	3.45	<0.01 **	20				
0.50	6.55	<0.01 **	18				
0.34	1.24	0.23	18				
0.30	3.36	<0.01 **	17				

Table 5.6 Gradients of regressions of ln TL on ln larval period for each of the high and low food cultures for *T. vittatus*. T-values test for difference from gradient of 0 (* = significant at 5% level, ** = significant at 1 %).

The gradients of the regressions of TL on larval period for the high food cultures are all greater than those in the low food cultures. So at high food levels, there is a tendency for those larvae with long larval periods to grow to a larger size than those that transform earlier. However, under conditions of restricted food, this size increase is not as pronounced and is possibly absent.

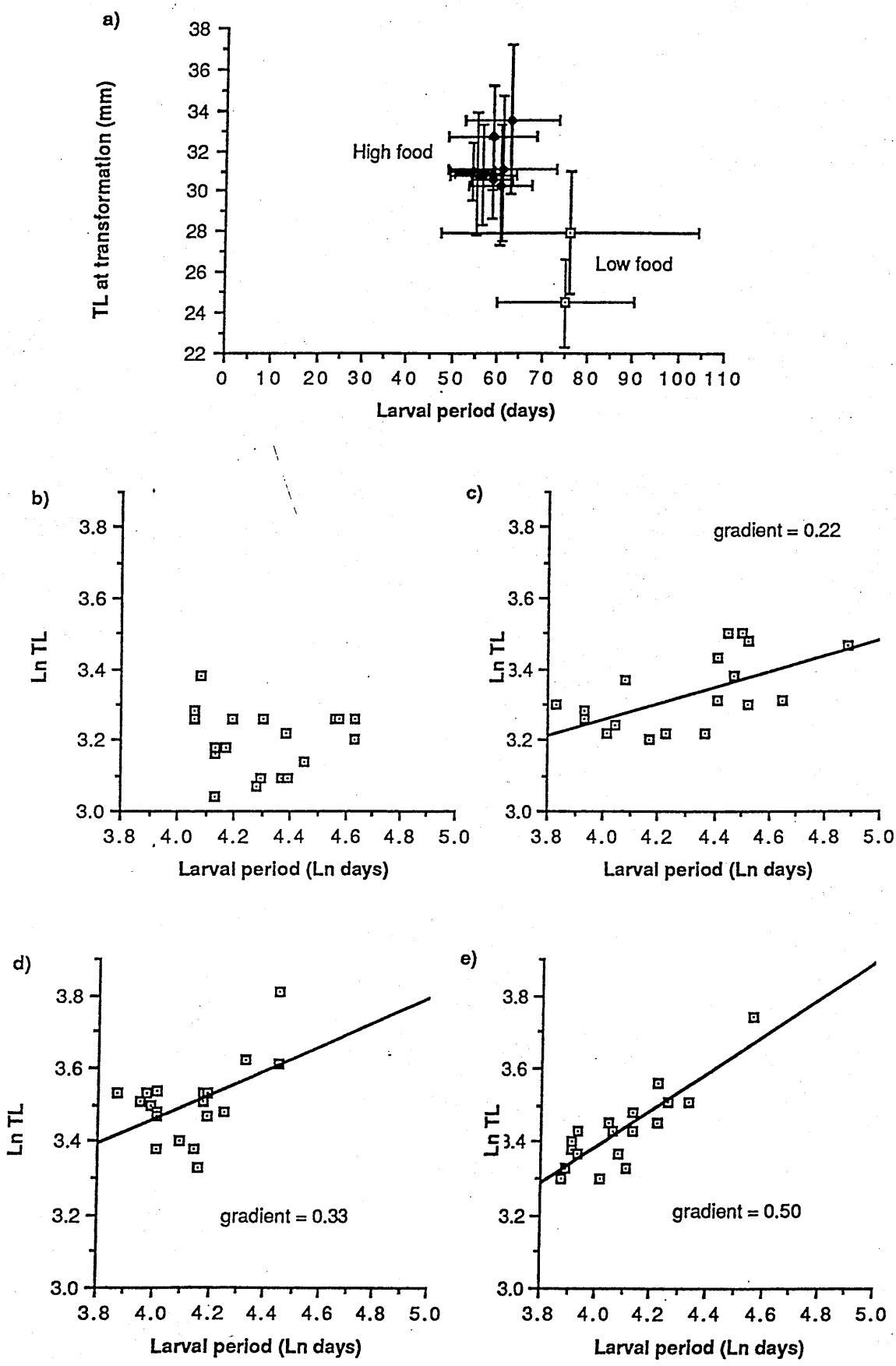


Fig. 5.2 Graphs to show effect of differing prey levels on metamorphs of *T. vittatus*. a) Size at (TL in mm) and timing of transformation. Means and standard deviations of 8 high and 2 low food cultures. b) and c) log TL plotted against larval period (log days) for the two low food cultures. d) and e) log TL plotted against larval period (log days) for two of the eight high food cultures.

T. vulgaris Body size (TL in mm) at transformation and larval period are presented in Table 5.7. The data of timing of transformation and TL at transformation were log-transformed to normalize positive skews in both of these parameters. These data are presented graphically in Fig.5.3.

Size (TL) at transformation Variances for Ln TL at transformation were considered to be similar, $F_{max} = 1.88$ (> 1.9 at 5% level, by extrapolation), so a one-way ANOVA was used to analyse these data. F-ratio = 11.795, $p < 0.001$. There is a significant effect of food on body size at transformation. Low and reduced feeding regimes produced smaller metamorphs than the high feeding regime.

Larval period Variances for Ln larval period were not found to be equal, $F_{max} = 4.31$ (> 1.9 at 5% level, by extrapolation) so a Kruskal-Wallis test was used to analyse these data. $H = 31.720$, $p < 0.001$. There is a significant effect of food level on larval period. Low and reduced food levels increase larval period.

Variance in body size Low and reduced food seems to have little effect on variation in body size at transformation (see coefficients of variation [CV] in Table 5.7. However, variation in length of larval period did increase with low and reduced food. Metamorph body size increases with larval period, but this rate of increase seems to be less at reduced and low feeding regimes.

Food level		High	Reduced	Low
	mean	29.1	25.9	26.1
	s.d.	3.49	2.598	3.567
TL	CV	12.02	10.02	13.65
	n	39	35	21
	var	12.24	6.75	12.72
	mean	57.4	73.77	96.95
	s.d.	10.27	21.82	37.33
Larval period	CV	17.88	29.57	38.51
(days)	n	39	35	21
	var	105.41	475.95	1393.7

Table 5.7 Mean body size at transformation and larval period for *T. vulgaris* larvae under high, reduced and low feeding regimes.

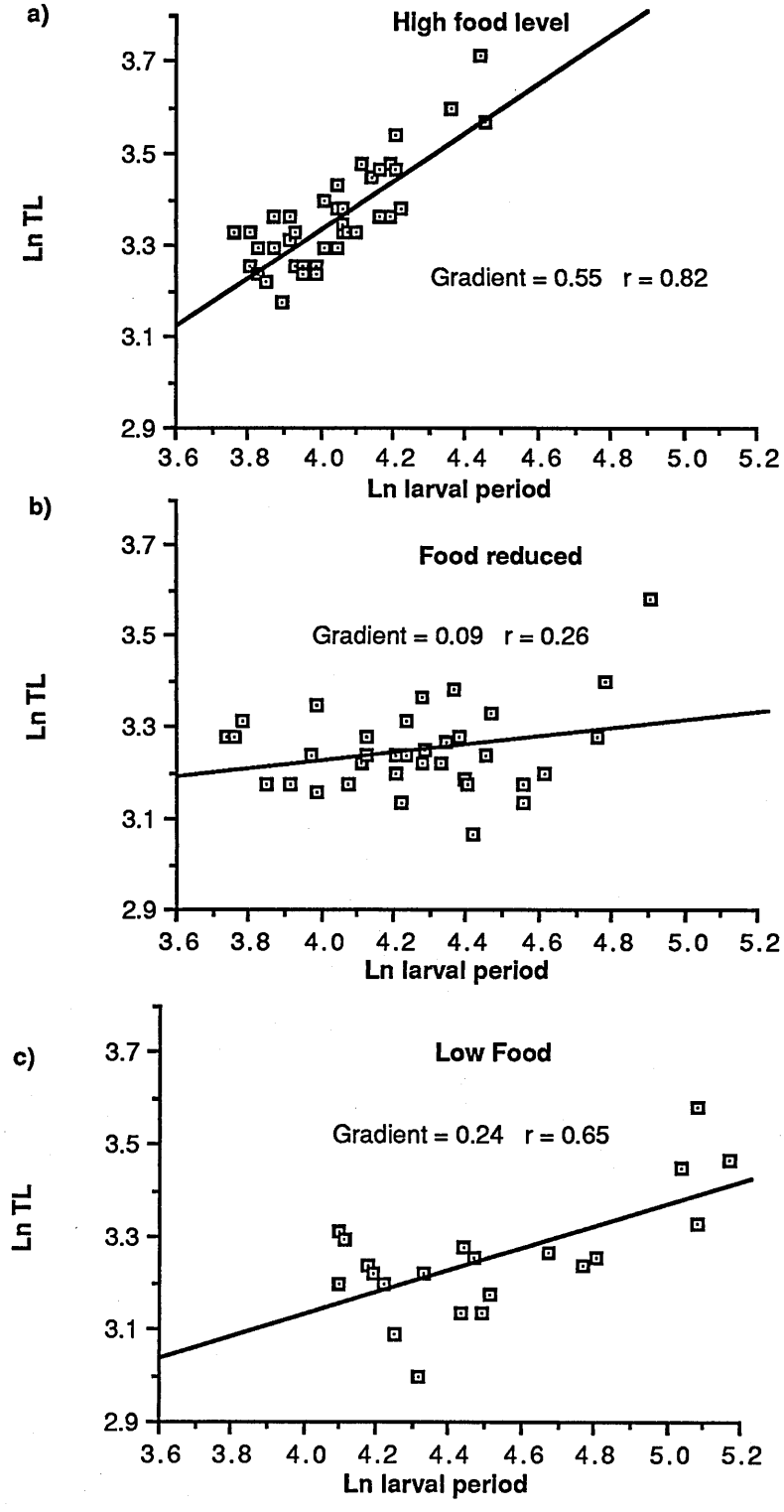


Fig. 5.3 Larval growth responses of *T. vulgaris* under three different feeding regimes. (a) High food, (b) reduced food, (c) low food.

Discussion The results for both species show that low food generally gives rise to smaller metamorphs, with longer larval periods. This is consistent with the growth responses shown by *Ambystoma texanum* (Petranka 1984) and *Taricha torosa* (Kaplan 1985). Some individuals under low food conditions are able to attain the same size as those under *ad libitum* conditions, but it takes longer to reach that size. The growth response of the reduced food *T. vulgaris* is slightly different, since the first few metamorphs appeared to show the same sort of growth characteristics as comparable larvae in the high food groups. It is the later metamorphs that show divergent body size and timing of transformation. This is presumably because the most rapidly developing larvae in the reduced food groups had almost completed larval development by the time that the food level was reduced. Food was reduced on day 35, and the first metamorph was removed on day 42. Hence these early metamorphs would have completed most of their larval growth under high food levels.

Both species show a gradual increase in metamorph size with increasing larval period. Collins (1979) proposed that increasing body size of metamorphs leaving a common environment could be due to a a competitive release mechanism, because if larvae were growing in a density-dependent manner, then the nature of the growth environment would improve with each successive larva that transformed. Hence he predicted that in competitive situations, body size of metamorphs would increase with larval period. However, Chapter 4 showed that *Triturus* metamorph body size tends to increase even when larvae are reared as singletons, so that the same pattern observed in the present study cannot be interpreted as being supportive of Collins's model. In the present study, the rate of increase of body size with increasing larval period tended to decrease at low food levels in both *T. vittatus* and *T. vulgaris*. This trend is the reverse of that predicted by Collins. However it does suggest that those larvae that have long larval periods may suffer from the ill-effects of low food levels to a greater extent than faster developing larvae, since under high food levels, long larval periods are accompanied by large size at transformation, but this advantage is decreased at low food levels.

The tendency for *T. vulgaris* larvae to show greater variation in larval period at these

low prey levels is suggestive of differential growth rates caused by low food levels. This may be generated by differential prey capturing abilities or differential assimilation and energy expenditure efficiencies at low food levels. However, variation in body size at transformation did not tend to increase, which suggests that larvae prolonged their larval periods in order to attain a certain size prior to transformation. Wilbur and Collins (1973) proposed that there is a lower size limit that must be attained to allow survival in the terrestrial form. The present study is not quite consistent with this hypothesis since metamorph size still tended to increase, although relatively little, with larval period.

The results of this study highlight three ways in which low levels of prey intake must be regarded as deleterious to the fitness of newt larvae. Low prey intake increases the length of time spent at a small size, which in the natural situation may increase the likelihood of predation (see Section 4.1). Low prey intake reduces body size at transformation, which is believed to be associated with reduced physiological and growth performance (see Section 4.1). Finally, low prey intake prolongs the length of the larval period, which will reduce the length of time during which it is possible to grow during the eft stage, prior to the first winter.

Conclusion In *T. vittatus* and *T. vulgaris*, low rate of prey intake causes a reduction in body size of metamorphs and a prolongation of larval period.

5.3 Effect of Larval Density on Larval Growth

Introduction The effects of larval density on growth of amphibian larvae have a long history of study (eg. Adolph 1931, Richards 1958, Smith 1983). Laboratory studies of anuran larvae show that increasing larval density generally increases larval period and decreases growth rates and size at transformation (eg. *Hyla gratiosa*, Travis 1983a, *Rana pipiens*, Gromko et al. 1973). The mechanisms underlying this effect are complex and are still debated. Anuran larvae may compete exploitatively for food (eg. Savage 1952) or more subtly via release of cells. Richards (1958) found single cells in the faeces of crowded larvae and concluded that it was these that inhibited growth, after ingestion by coprophagic larvae. However, Gromko et al. (1973) found that coprophagia was not necessary to generate the typical effects of crowding in the laboratory growth of *Rana pipiens*. In fact they found that access to faeces increased growth rate, suggesting that coprophagia may be a beneficial feeding strategy. They concluded that stress mediated the crowding response, relegating Richard's cells to a parasitic role that, although capable of reducing growth, were not in fact the mechanism of competition. They were not able to find any cells in faeces from their larvae.

Anuran larvae may also be able to inhibit the growth of others via dissolved substances. In laboratory studies of *Rana utricularia*, Steinwascher (1978) found that water conditioned by larvae inhibited the growth of conspecifics. This effect is not seen in all species, and was not detectable in the experiments of Gromko et al. described above.

Urodele larvae show the same growth responses to larval density, but there is less information available concerning the mechanism of intraspecific competition. For example, correlative evidence of density-dependent growth in urodele larvae has been reported. Kusano (1981) found that size at transformation was inversely related to larval density at the end of the larval period in *Hynobius nebulosus*. Morin (1983) found that higher numbers of *Notophthalmus viridescens* efts, collected from artificial ponds lead to a decrease in metamorph mass. Walls and Jaeger (1987) found that the larvae of *A.*

talpoideum and *A. maculatum*, showed a negative but not significant relationship between final density and body size in laboratory replicates. In the newt, *Notophthalmus viridescens dorsalis*, Harris (1987) found that growth rate in a cattle trough experiment was negatively correlated with final density and Harris et al. (1988) noted that the size at metamorphosis, from a natural pond, between years, was inversely related to larval density. Metamorphs of this same species have also been found to vary in mean body size, in an inverse relationship to the numbers emerging from cattle-troughs in a study by Alford (1989).

Experimental evidence of density-dependent growth in urodele larvae may be difficult to obtain for practical reasons. For example, Harris in his 1987 experiment (see above) manipulated initial larval density to examine its effect on growth. However, random mortality between replicates, during early larval life, produced the result that there was no effect of initial density on growth; however final density was negatively correlated with larval growth rate. Despite this, there is some experimental evidence of density-dependent growth in urodele larvae. Stenhouse et al. (1983) recorded density-dependent effects on growth of *A. maculatum* in the laboratory, and *A. opacum* in the laboratory and in field enclosures. Semlitsch (1987a) reared *A. talpoideum* at various densities, within the range of densities noted in a natural situation, in artificial ponds (cattle troughs) and recorded density effects on body size and survival. The general results of these experimental studies are that increased larval densities tend to prolong larval period, reduce larval survival and produce smaller metamorphs.

The study of density effects using artificial ponds and enclosures has been criticized on the grounds that such experiments do not necessarily mimic true pond conditions, in that larval density may be artificially high and food levels too low (Petranka and Sih 1986). However, whole pond manipulations and the use of large enclosures yield similar results to the experiments summarized above, which suggests that the results from small scale manipulations of larval density may well be valid. Petranka (1989) used fences to divide ponds into two halves, enabling him to reduce initial larval density of *Ambystoma opacum*

in one half of each of a series of natural ponds. Body size of metamorphs was negatively correlated with larval density. Scott (1990) used large field enclosures to manipulate the density of larval *A. opacum*, with the result that high density decreased growth rate and body size at transformation, increased larval period and decreased survival.

The mechanisms of competition are not always apparent, particularly in the case of correlative evidence, but it is possible to speculate on the mechanisms of crowding effects that may be relevant to urodele and *Triturus* larvae in particular. Urodele larvae are carnivorous and are not known to eat faeces. Hence growth inhibition via coprophagia and consequent intake of inhibiting cells would be unlikely to occur in *Triturus*. Bell (1974) performed rearing experiments on *T. vulgaris* larvae using water conditioned by high densities of conspecifics and could detect no effects of growth inhibition, which suggests that dissolved substances play no part in mediating growth inhibition in this species. Walls and Jaeger (1989) came to the same conclusion concerning *Ambystoma maculatum* and *A. talpoideum* grown in laboratory experiments. So, if *Triturus* larvae do in fact show density-dependent growth it must be mediated either through exploitative competition for food or by behavioural and/or stress related mechanisms. Semlitsch (1987a) noted that the *A. talpoideum* grown at high densities suffered more physical damage than those at low densities, which suggests that aggressive encounters increased with higher larval densities. The work of Walls and Jaeger (1987 and 1989) shows that Ambystomatid species demonstrate both types of process under laboratory conditions, but Petranka's (1989) experiments suggest that aggression is involved rather than exploitative competition for food. Smith's (1990) laboratory studies of *A. opacum* also suggest tht aggressive interactions mediate density effects. He found that when a size difference existed between two larvae, then the larger negatively affected the growth of the small larva. However, this effect was removed if the two larvae were not allowed to physically interact, but still shared a food source and water environment.

Urodele larval competition may also take a more subtle form. The study of Harris et al. (1988) provides evidence of spatial segregation of larvae by size, the implication being

that since larvae of different sizes compete, they tend to avoid larvae of a larger size class. Hence smaller larvae may be forced into a less favourable microhabitat, which could adversely affect growth and/or survival. So, it would seem that density-dependent growth in urodele larvae is likely to be regulated by different mechanisms to those proposed for anurans, with perhaps the exception of behaviourally mediated competition.

Competitive interactions among fish were thought only to occur when food is limiting eg. Magnuson (1962) found that medaka, *Oryzias latipes*, only showed the effects of competition, in the laboratory, when food was limiting (and see Weatherley and Gill, 1987). However, there is a body of work that shows that fish can demonstrate the effects of competition, such as growth depensation (an increase in variance in body size with time due to differential growth rates [Magnuson 1962]) even when food is unlimited, but individuals are nevertheless crowded (Brown 1946, Abbott and Dill 1989) which suggests that social interactions may be involved in intraspecific competition in fish.

Previous studies of competition in larval *Triturus* have focussed on interspecific relationships (eg. Avery 1968, Szymura 1974 and Dolmen 1983). Some species do occupy different niches. Szymura and Dolmen showed that larvae of different species occupied different microhabitats (*T. alpestris*, *T. cristatus*, *T. montandoni*, and *T. vulgaris* in the former study and *T. cristatus* and *T. vulgaris* in the latter). However, Avery found that the larvae of the three British species fed on similar prey items. Szymura and Dolmen's conclusions were that spatial segregation was a means of reducing competition for food, and Avery concluded that there was potential for competition for food. However, these studies do not actually demonstrate that there is competition for food. Dietary overlap is insufficient evidence for the existence of interspecific competition for food (Colwell and Futuyma 1971) and Griffiths (1987) proposes that newt populations may exist at levels below the carrying capacity of their environment, so that prey is not a factor limiting population size.

The present study adopts a different approach. There is a mounting volume of evidence that urodele larvae do in fact show density-dependent growth under certain conditions. This being so, the present study examined the effect of differing larval

densities on the growth of larvae from two species (*Triturus vittatus* and *T. vulgaris*) grown under laboratory conditions, to ascertain whether *Triturus* larvae have the potential to express density-dependent growth.

Methods

Triturus vittatus:

Initially, twelve cultures of larvae were reared as described in section 5.2. (25 larvae per 4 litres of water, fed on an *ad libitum* basis). Eight cultures were assigned to 'high' density and four to 'low' density. At five weeks the larval populations of the cultures were reduced to create the two experimental larval densities. The eight high density cultures were reduced to 20 larvae per container and three low density cultures were each split into two separate containers to create densities of eight larvae per culture (all larvae in one of the cultures allocated to low density died prior to this stage). Note that the 'high' food cultures from the experiment in section 5.2 were used in the present experiment as the 'high' density condition. Date of and body size at transformation were recorded as in General Methods, Section 4.2.

T. vulgaris

Ova and hatchlings were treated as described in Section 4.2. At Gallien and Bidaud stage 40, six rearing containers (26 x 14 cm) filled with 2 litres of conditioned tap water were stocked with two different larval densities. Three cultures were stocked with 10 larvae (low density) and three cultures were stocked with 20 larvae (high density). These cultures were maintained in a laboratory as described in 5.2 and fed on an *ad libitum* basis. Larvae were measured once a week and each metamorph was measured at transformation, noting date of this event.

Results

T. vittatus

Mean body sizes (TL), larval periods, standard deviations and coefficients of variation of metamorphs from each culture are shown in Table 5.8. T-tests were performed to compare the mean values of body size, larval period and coefficients of variation, for both of these, of the high density cultures with the means of the low density cultures. The results of these comparisons are presented in Table 5.9. There was a significant difference in body size (TL) between the two densities. High densities produced smaller metamorphs ($t = 3.60$, $p = 0.004$, 12 d.f., see Fig. 5.4 a). There was a trend towards a greater size variance (CV) at higher densities, but this was not statistically significant ($t = 1.81$, $p = 0.095$, 12 d.f., see Fig. 5.4 a). High densities did not significantly increase mean larval period ($t = 0.384$, $p = 0.384$, 12 d.f., see Fig. 5.4 b), but they did significantly increase the variance in larval period ($t = 2.24$, $p = 0.045$, 12 d.f., see Fig. 5.5 b).

High Density cultures						
TL at transformation (mm)			Larval period (days)			
Mean	s.d.	CV	Mean	s.d.	CV	n
33.5	3.68	10.99	62.7	10.48	16.7	19
30.8	2.46	7.99	56.5	7.44	13.18	20
30.3	2.94	9.71	60.2	7.07	11.76	19
30.6	1.96	6.39	58.8	4.70	7.99	17
31.0	1.41	4.55	54.1	4.77	8.82	20
31.1	3.54	11.39	60.7	12.05	19.96	18
30.9	3.04	9.86	55.2	4.95	8.97	18
32.6	2.58	7.90	58.6	9.95	16.97	17
Low Density cultures						
TL at transformation (mm)			Larval period (days)			
Mean	s.d.	CV	Mean	s.d.	CV	n
33.1	1.90	5.74	58.0	5.55	9.58	8
33.8	2.91	8.62	59.4	6.57	11.06	8
32.4	2.99	9.22	59.2	5.78	9.76	5
34.1	1.72	5.05	56.1	4.16	7.40	8
32.9	1.35	4.09	53.9	2.85	5.30	7
32.8	1.91	5.83	55.8	4.95	8.88	8

Table 5.8 Mean body sizes and larval periods of the 14 cultures of *T. vittatus* reared at either high or low larval density. Standard deviations (s.d.), coefficients of variation (CV) and numbers of metamorphs emerging per culture (n) are also given.

	Density	Mean	s.d.	t-value	p	d.f.
Body size (TL)	High	31.35	1.11	3.60	0.004*	12
	Low	33.18	0.64			
Larval period	High	58.35	2.92	0.090	0.384	12
	Low	57.07	2.16			
CV (body size)	High	8.60	2.34	1.81	0.095	12
	Low	6.43	2.04			
CV (larval period)	High	13.05	4.43	2.24	0.045*	12
	Low	8.66	2.04			

Table 5.9 Body size, larval period and coefficient of variation for *T. vittatus* larvae grown under two different larval densities. * Indicates significant difference between two densities at 5% level of probability.

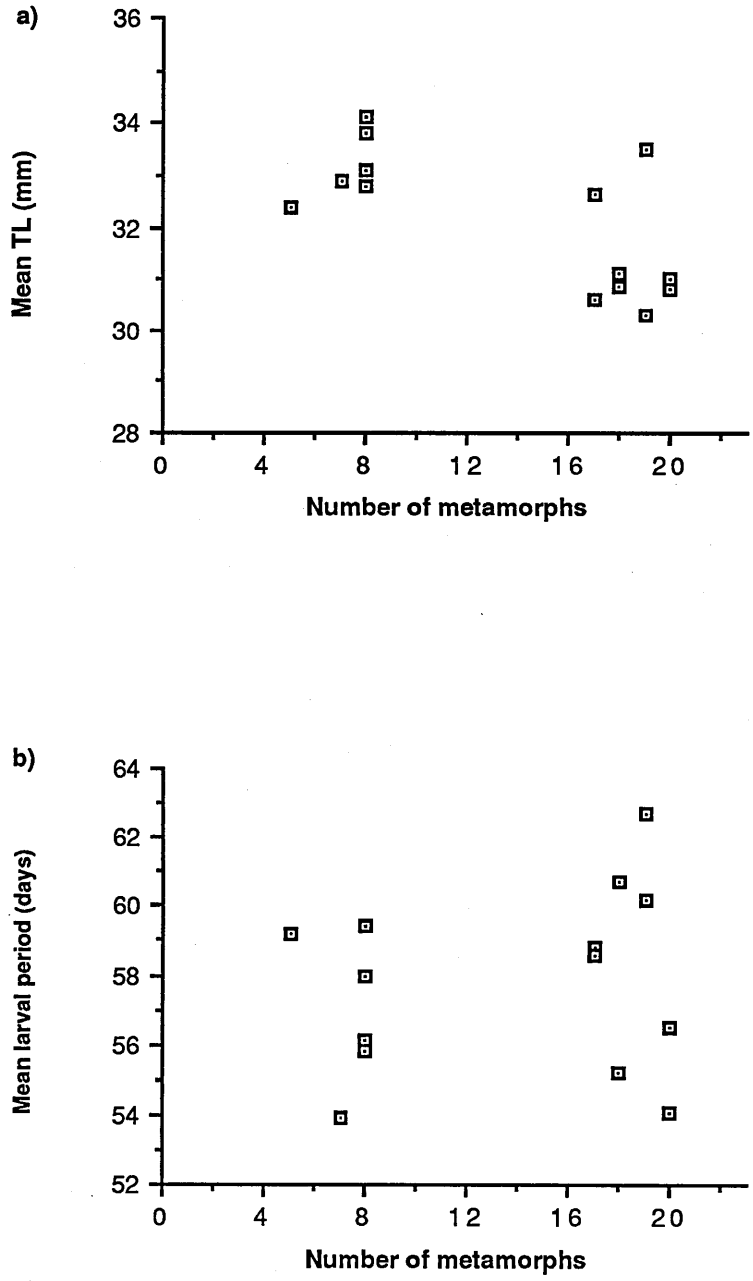


Fig. 5.4. Graphs to show effect of larval density on metamorphs of *T. vittatus*. (a) Mean body size (TL) plotted against number of metamorphs per culture. (b) Mean larval period plotted against number of metamorphs per culture.

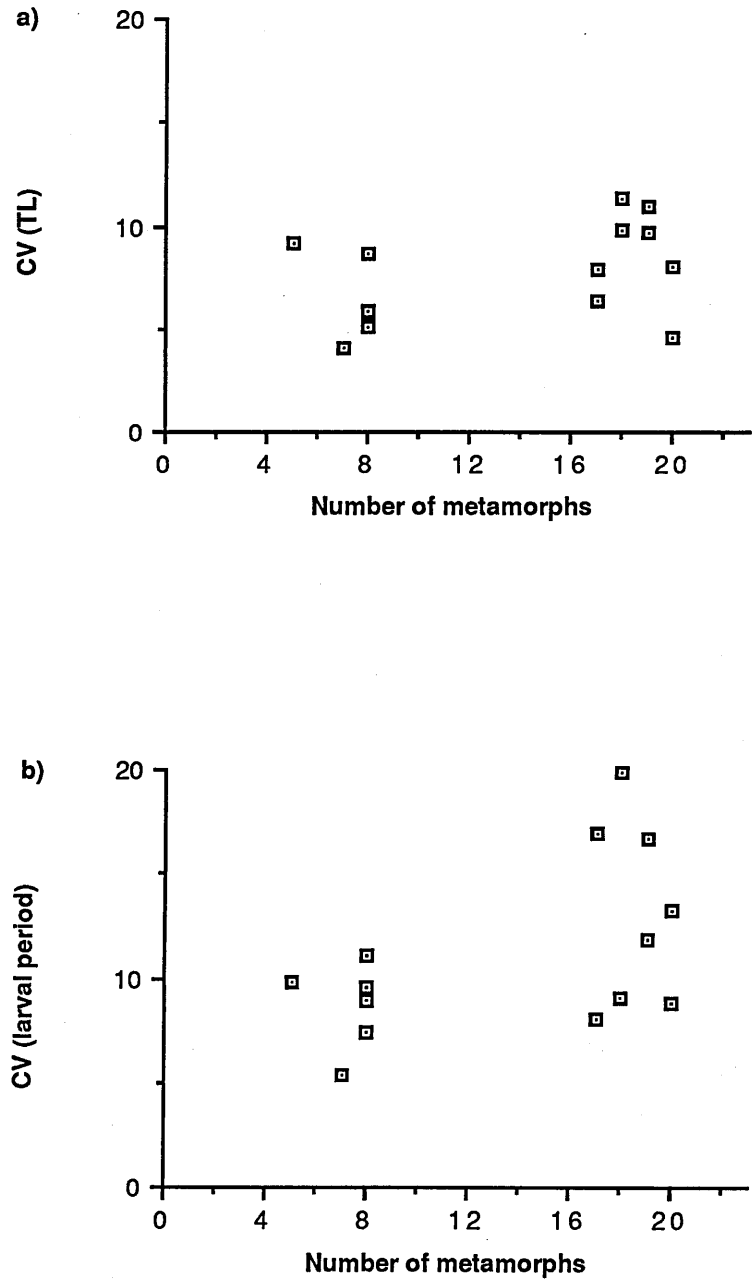


Fig. 5.5. Graphs to show effect of larval density on variation in body size and length of larval period in *T. vittatus*. (a) Coefficient of variation of TL plotted against number of metamorphs per culture. (b) Coefficient of variation of larval period plotted against number of metamorphs per culture.

T. vulgaris

Since only three cultures of larvae were reared to transformation at the two different larval densities, it is impossible to make statistical comparisons between the mean values for each culture. Hence larval periods and size at transformation of each individual larva were pooled within each of the two rearing regimes. T-tests were used to compare body size at transformation (TL) and larval period between the two densities. Larvae grown at high density had longer larval periods than those grown under low density, $t = 6.658$, $p < 0.001$, 78 d.f., but there was no difference in TL at transformation under the different densities, $t = 0.43$, $p = 0.67$, 78 d.f. See Table 5.10.

	Density	Mean	s.d	CV	t-value	p	d.f.
Body size	High	31.4	3.52	11.22	0.43	0.67	78
	Low	31.0	2.53	8.15			
Larval period	High	58.1	9.21	15.85	6.658	<0.001*	78
	Low	46.8	4.22	9.02			

Table 5.10 Mean values, standard deviations and coefficients of variation for *T. vulgaris* larvae grown under two different larval densities. * Indicates significant difference between the two densities at 5% level of probability.

Larval growth curves are shown in Fig. 5.6(a). It can be seen that the growth curves of the cultures reared at the two different densities cluster out into two discrete growth trajectories. Variance in body size is shown in Fig. 5.6(b). At low densities, coefficients of variation remained fairly constant over the course of the rearing trial, peaking between values of five and eight. At high densities the coefficients of variation rose sharply as larvae grew, peaking between values of fifteen and twenty, but falling off again towards the end of the span of larval periods.

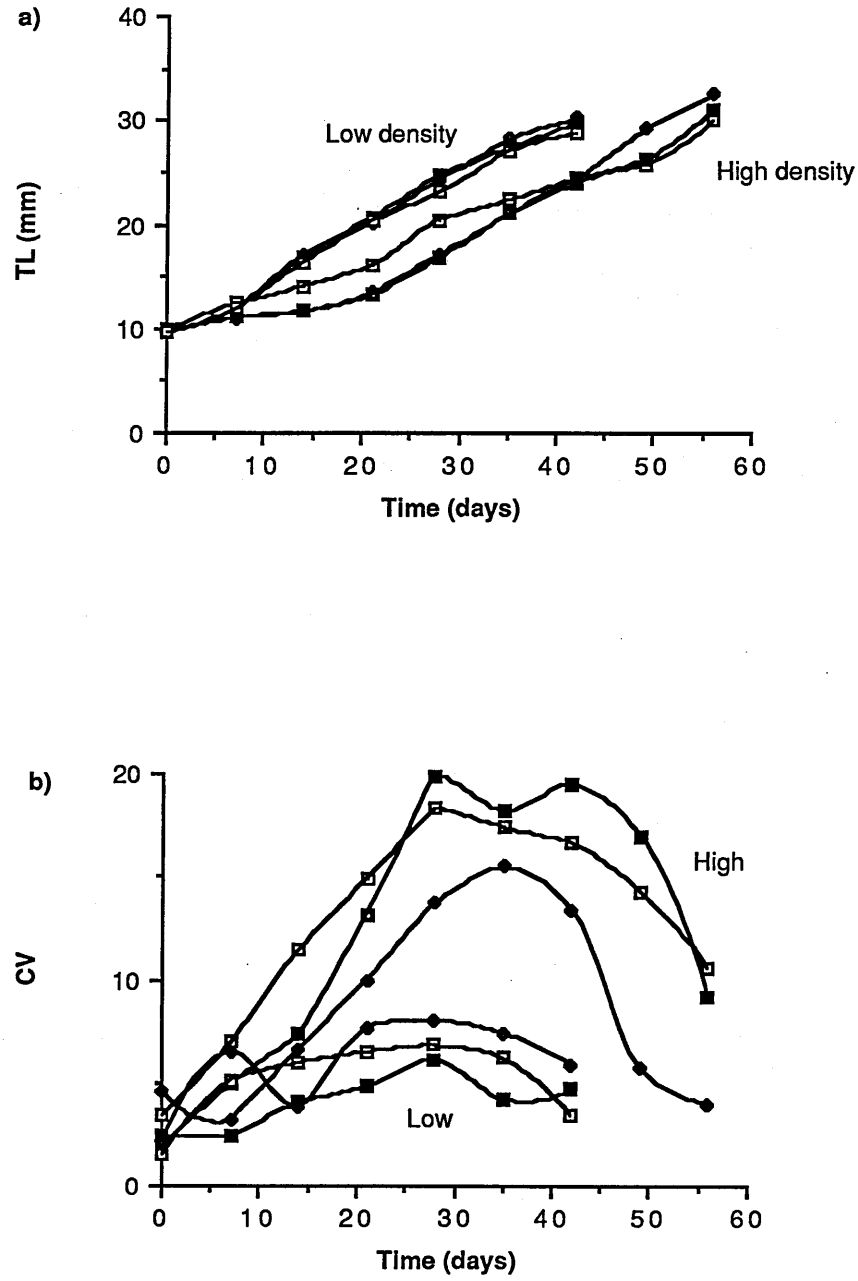


Fig. 5.6. Graphs to show effect of larval density on growth of *T. vulgaris* larvae. (a) Mean size of larvae in each culture plotted against time, (b) coefficients of variation of body size (TL) of larvae in each culture plotted against time.

Discussion The effects of density on larval growth and size at transformation recorded here are typical of density-dependent growth seen among other amphibian species in laboratory and field enclosure experiments, and agree with correlative evidence from natural ponds. However, laboratory studies of larval growth are open to the same sort of criticism that has been levelled at the use of artificial ponds and small enclosures, namely that larval densities may be artificially high and hence food levels unnaturally depressed (Petranka and Sih 1986). Larvae in the present experiment were fed on an *ad libitum* basis so that the problem of food levels being overly depressed has been countered. Hence, it is possible to conclude that *Triturus* larvae have the potential to express density-dependent growth, even when food is not limiting, and this effect will be of direct relevance to future studies of larval growth in the laboratory.

In *T. vittatus*, high larval density led to smaller body size at transformation, with a trend towards increasing size variation. Mean larval periods were similar between the two densities, although variation in larval period within a culture increased at high densites, which means that some *T. vittatus* responded to high larval density in the typical manner of lenghtening larval period, but others must also have been able to decrease the length of time that they spent in the larval stage.

In *T. vulgaris* high larval density increased larval period but had little effect on the final body size of the metamorphs. The variation in body size, within cultures, recorded in *T. vulgaris* during larval growth needs some explanation. An increase in variation in body size within a cohort of growing organisms may be expected in situations where individuals compete (Lominicki 1988). Hence the lack of increase in the coefficient of variation in the low density cultures suggests that larvae were not competing whereas the rapidly increasing coefficients of variation in the high density cultures (Fig 5.7 b) are suggestive of competitive interactions producing rapidly increasing variation in body size with time. The decrease in coefficient of variation seen in the latter cultures towards the end of the span of larval periods is not typical of organisms growing under competitive situations, but can be attributed to the fact that some of the smaller larvae were transforming over this period, thus reducing the variation in body size of the remaining larvae.

In both species it is apparent that the effect of increased larval density on variation in body size at transformation is not as great as the effect on variation in timing of transformation. In *T. vittatus* coefficients of variation in body size remained constant between densities whilst coefficients of variation for larval period increased at high densities. In *T. vulgaris* coefficients of variation only increased from 8.15 to 11.22 for body size but from 9.02 to 15.85 for larval period. So it would seem that at high densities, some larvae tend to prolong larval period rather than suffer reduced body size. It is equally possible that the later transforming larvae experience a release from the negative effects of high larval density, as their earlier transforming peers leave the larval growth environment. This may explain why no size difference was observed between the two densities of *T. vulgaris*. Those larvae that suffer little due to crowding, transform as they would at a low density, whilst those that are adversely affected show reduced growth during early larval life, but are then able to become large metamorphs as their competitors leave the water.

There is no documented evidence that *Triturus* larvae exhibit density-dependent effects in natural populations, but the literature reviewed in the introduction strongly suggests that, in some species of salamandrids, density-dependent larval growth does occur. Since there are ecological similarities in the lifestyle of lenthic salamandrid larvae, of different species, it would be reasonable to predict that a future study of *Triturus* larval growth in the field would demonstrate density-dependent growth in some situations.

Population control through larval interactions Savage (1952) proposed that competition in the larval stage could regulate amphibian population sizes This could occur through two mechanisms. First, by directly affecting population size, so that competition at high larval densities reduces the survival of metamorphs, which leads to reduced adult recruitment. Second, in species in which fecundity is related to body size, competition could regulate population size through its effect on adult body size. For example Baur's (1988) study of natural populations of the snail *Arianta arbustorum* found that, at high densities, mucous trails slow activity of juveniles and inhibit growth rate, producing smaller adult females

with lower fecundity. Semlitsch (1987a) found a similar effect in his experiments on paedomorphic *A. talpoideum* grown in 'cattle troughs'. High larval densities produced smaller females with lower fecundities. The above two mechanisms need not be mutually exclusive. From the patterns of growth shown by *T. vittatus* and *T. vulgaris* in the present study it would seem that these larvae could potentially be subject to population regulation via density-dependent effects in the larval stage. The reduced body size of *T. vittatus* and the reduced growth rate of *T. vulgaris* at high densities could be translated into either smaller adult body size, or reduced juvenile survival, as outlined in Chapter 4.1.

The present study shows that *Triturus* larvae have the potential to exhibit negatively density-dependent larval growth in food unlimited situations. Therefore larval growth does have the potential to regulate population size as Savage (1952) suggested. However, urodele population regulation as a density-dependent processes in the field may involve different processes to those in anurans, especially if prey becomes a limiting factor. When Savage postulated that competition during the larval phase may regulate amphibian populations, he had temperate anurans in mind rather than urodeles. His focal species, *Bufo bufo* and *Rana temporaria*, exhibit a life cycle that discretely divides the larval and post-larval stages into two different ecological niches. The larvae occur at relatively high densities and may compete for food in ponds, whereas the post-larval stages feed on land, at low population densities and are hence unlikely to compete for food. However larval and post-larval urodeles may show great feeding niche overlap. The aquatic adults feed on live prey items in ponds, just as the larvae do. Verrell (1987) noted that most of the annual growth of adult smooth newts occurs whilst they are in the aquatic phase, so they may be using ponds as an important source of food. Hence, any appraisal of exploitative competition for prey items in ponds as a mechanism of population regulation in urodeles should note that aquatic adults may be involved as well as larvae. Gill (1979) presented data to suggest that adult *Notophthalmus viridescens* show density-dependent effects. Manipulations of numbers of adults in natural breeding ponds showed that female annual survival was negatively correlated with population density and females from increased

density populations tended to show reduced body weights. Morin (1973) found correlative evidence of competition between the adult and larval stages of this species. In his 'cattle trough' experiments, the number of emerging efts was negatively correlated with eft weight and adult weight increase during the aquatic phase. Hence, if prey becomes a limiting factor in newt ponds then population regulation may occur via adult as well as larval interactions.

Although the present study suggests that newt larvae have the capacity to express density-dependent growth in food unlimited situations, this does not imply that natural populations are regulated in this manner. Results from studies of other urodele species show that as well as being subject to density-dependent regulatory processes (*sensu* Nicholson and Bailey [1935]), larval urodele populations also seem to be affected by density-independent mechanisms (*sensu* Andrewartha and Birch 1954). For example, pond desiccation has been recorded as being the major factor affecting growth and survival of larval *Ambystoma tigrinum* (Semlitsch 1983) and growth of larval *A. talpoideum* (Semlitsch et al. 1988), and predation by fish has been found to determine survival in *A. maculatum* (Ireland 1989). Hence it seems that urodele larvae may express density-dependent growth, but only under certain conditions (Semlitsch 1983).

Conclusion The two species responded to changes in larval density in different ways. At high density, *T. vittatus* showed a decrease in body size of metamorphs, but no change in larval period. *T. vulgaris* responded to high density by increasing larval period, but maintaining metamorph size. In both species high larval densities tended to increase variation in larval period, but not in mean body size.

5.4 Maternal Effects on Larval Growth

Introduction Differences in larval growth or survival that are engendered by differences in the quality of the eggs from which those larvae arose fall into two categories, genetic or maternal. Genetic control of larval growth characteristics has been demonstrated by Travis (1981) who found that half sibships of *Pseudacris triseriata* differed in growth parameters, although this effect is often not as great as that of maternal factors. Maternal effects can be more apparent than genetic effects as shown by non-genetically based differences in growth rates between larvae from different females (Travis 1981) and by differences in larval period attributable to differing egg size between females (Berven 1982). Maternal effects are those generated by phenotypic differences in ovum quality, for example large ova have been shown to produce faster-growing larvae, presumably because large ova have greater energy invested in them by the mother (see Ch 2 discussion and Chapter 3.1). Travis also points out that maternal and genetic control may also interact (ie. genetic control over ovum quality) and the environment in which an ovum develops, that is to say its mother, will in turn be a phenotypic expression of her own genotype.

This section examines data collected during the course of this project to test the hypothesis that maternal effects, mediated through ovum size, affect subsequent larval growth.

For the present study it was not possible to relate ovum size to any larval parameter. This was because the ovum jelly coat acts like a lens, increasing the apparent size of the ovum. Since the shape of the jelly coat is not regular it cannot be assumed that all ova are magnified to the same degree. Hence it was necessary to remove each ovum from its jelly coat in order to measure it. This operation always resulted in the destruction of the ovum itself, unless the ovum was fixed in 4% formalin. Hence, no hatchlings could be obtained from ova of known sizes. As an alternative measure of maternal investment, larvae were measured immediately after hatching. This was considered to be a valid measure because at this stage the larvae have not captured any prey items, so all growth up to this point will

reflect maternal investment in the ovum. Size of hatchlings may be an even more valid measure of maternal investment, since it is the larvae that have to deal with the problems of the environment that may affect them in a size-related manner. The ova, being sedentary and wrapped in the leaves of pond weed, are relatively well-protected against these problems. Also Kaplan (1980, 1985) has shown that there is a positive relationship between ovum size and hatchling size in *Ambystoma opacum, A. maculatum A. tigrinum* and *Taricha torosa.*

Methods This study analyses data drawn from groups of larvae reared in other sections of this thesis. The larvae in question are described in Table 5.4.1. These larvae were reared individually, at one of four temperatures, and fed *ad libitum.* Descriptions of collection and maintenance of ova and larvae are given in the relevant chapters of this thesis.

Label	Description	Temperature	Reference
Tc	*T. cristatus* (Milton Keynes)	20°C	Ch. 4
TvW	*T. vulgaris* (Woolmer)	25°C	Ch. 6
Tv25	*T. vulgaris* (Milton Keynes)	25°C	Ch. 5
Tv20	*T. vulgaris* (Milton Keynes)	20°C	Ch. 4, Ch. 5
Tv15	*T. vulgaris* (Milton Keynes)	15°C	Ch. 5

Table 5.11 Larvae used in study of maternal effects on larval growth. 'Label' refers to name given to group of larvae for present study. 'Description' provides species and locality of origin. 'Temperature' refers to temperature during rearing study. 'Reference' indicates which part of thesis contains full description of rearing study.

The data collected for analysis in this study were:

Body size (TL) at Gallien and Bidaud stage 40 (hatchling).

Body size (TL) at Gallien and Bidaud stage 53.

Body size (TL) at Gallien and Bidaud stage 55c (transformation).

Time taken (days) to reach the above stages 53 and 55c.

Growth rate (mm/day) over period needed to reach stages 53 and 55c.

The hypothesis adopted for the analysis of these data was that larger hatchlings (stage 40) would either produce larger larvae, or metamorphs, or faster growing or developing larvae. These hypotheses were tested using Pearson product-moment correlation coefficients to find whether there was a significant, positive correlation between size of individuals at stage 40 and six parameters of growth/differentiation:

1. Size (TL) at stage 53.
2. Time to attain stage 53.
3. Growth rate (change in TL/day) to stage 53.
4. Size (TL) at transformation.
5. Time to attain transformation.
6. Growth rate (change in TL/day) to transformation.

Correlation coefficients are presented in Table 5.12.

Label	Larval growth parameter	r	n
Tc	Size at stage 53	+0.538 *	19
	Time to stage 53	-0.274	19
	Growth rate to stage 53	+0.449	19
	Size at transformation	+0.049	18
	Larval period	-0.169	18
	Growth rate to transformation	+0.130	18
Tv25	Size at stage stage 53	+0.550 *	14
	Time to stage 53	+0.029	14
	Growth rate to stage 53	+0.402	14
	Size at transformation	+0.439	13
	Larval period	-0.214	13
	Growth rate to transformation	+0.654 *	13
Tv20	Size at stage 53	+0.288	16
	Time to stage 53	-0.299	16
	Growth rate to stage 53	+0.476	16
	Size at transformation	+0.210	16
	Larval period	+0.002	16
	Growth rate to transformation	-0.002	16
Tv15	Size at stage 53	-0.349	14
	Time to stage 53	+0.219	14
	Growth rate to stage 53	-0.608 *	14
	Size at transformation	Not reared to transformation	
	Larval period	Not reared to transformation	
	Time to transformation	Not reared to transformation	
TvW	Size at stage 53	+0.260	19
	Time to stage 53	-0.108	19
	Growth rate stage 53	+0.392	19
	Size at transformation	-0.216	19
	Larval period	-0.305	19
	Growth rate to transformation	+0.201	19

Table 5.12 Pearson product-moment correlation coefficients between size at hatching and 6 parameters of larval growth or development. * Indicates significance at the 5% level.

The only significant correlations are between size at hatching and size at stage 53 in *T. cristatus*, size at stage 53 in *T. vulgaris* reared at 25°C, growth rate to transformation in *T. vulgaris* reared at 25°C and growth rate to stage 53 in *T. vulgaris* reared at 15°C.

Discussion Since there are in fact 27 correlations performed in this analysis, then some significance levels would be expected to be obtained by chance. The only significant relationship that occurs in more than one group of larvae is size at hatching and size at stage 53 (for *T. cristatus* and Tv25). This relationship is also positive for Tv20 and TvW, but negative for Tv15. These may be real effects reflecting the different effects of temperature or differences between populations or species, or it may reflect the fact that under laboratory rearing conditions there is no effect of size at hatching on subsequent larval growth. However, in view of the large number of correlation analyses performed, it appears that size at hatching has little real effect on subsequent growth parameters, but there may be a tendency for bigger hatchlings to maintain their size advantage to stage 53, although by the time of transformation, the latter effect of size at hatching has disappeared.

Ovum size effects have not been reported for all species, for example *Ambystoma maculatum* shows no relationship between ovum size, hatchling size and subsequent growth rate (Walls and Altig 1986). Similar results have been obtained by Crump (1984) who grew *Hyla crucifer* to transformation in the laboratory to compare larvae from small eggs with larvae from large eggs. She found that although larger eggs produced larger hatchlings, the size difference was not maintained to transformation and there was no detectable difference in larval period. The reason that no egg-size effect on growth was detected may be due to lack of environmental stress in laboratory rearing situations. Crump stated that she had created ideal growth conditions for the larvae. Travis (1983b) has noted that laboratory conditions do not emphasize differences in quality between individuals to a great an extent as do field conditions and Wootton (1990) notes that the relationship between fish egg size and subsequent growth and survival may disappear when unlimited food is available. The conditions under which the newt larvae of the

present study were grown were probably 'good'. There was no shortage of food, no predation and no exposure to the dangers of pond desiccation. So, although maternal effects on larval growth were not consistently evident in the present study, it would be unwise to conclude that hatchling size is an irrelevant life history parameter in terms of fitness and survival in a natural environment.

The work of Williamson and Bull (1989) may provide an alternative explanation for the lack of hatchling size effects on larval growth. They report that in the frog, *Ranidella signifera*, maternal effects were evident within a clutch, since egg size was positively related to hatchling size. However, between clutches, there was no egg size-hatchling size relationship. It appears that genetic and/or non-size related maternal effects influenced growth up to the hatching stage to a greater extent than egg-size alone. The groups of larvae in the present studies were of different parentages, and so if hatchling size effects only occurred within a clutch, then no effect of hatchling size would be detectable.

Conclusion Maternal influence, in terms of the size of the hatchling, may have a weak effect on size during the early stages of development, but these size differences diminish as development proceeds, so that maternal influence has no effect on timing of, or size at transformation.

5.5 Factors not examined in the present project:

There are other factors that may affect the growth of newt larvae that were not examined in the present study, but have been identified in other studies of amphibian and fish growth. A list and brief review of such factors is given below:

Oxygen concentration

Cannibalism

Genetic Inheritance

Size difference competition

Predation and higher order interactions

Oxygen concentration Levels of dissolved oxygen can limit fish growth (see Weatherley and Gill, 1987). Although level of dissolved oxygen is known to affect the behaviour of amphibian larvae, for example *Ambystoma tigrinum* takes more air from the surface at lower oxygen concentrations (Wassersug and Seibert 1975), the effect on growth has not been investigated. It does not seem to affect the rate of transformation of *Ambystoma tigrinum* (recorded via reduction in larval tail depth [Schrode 1972]). In the present series of studies, the effect of dissolved oxygen was not investigated or controlled for. It was assumed that levels of dissolved oxygen varied little between beakers or cultures in any single study.

Cannibalism/Interspecific predation It is often assumed that urodele larvae will compete via cannibalism, but this type of predation may not be prevalent in all populations. For example Verrell (1985b) in an analysis of the stomach contents of 126 aquatic adult *T. vulgaris* found that none contained conspecific larvae and only three contained ova. Moreover, there is little empirical evidence to validate larval-larval predation. Avery (1968) did not record cannibalism among larvae, and neither did Bell (1975). Harrison (1985)

only found one newt larva in a stomach analysis of 205 *Triturus* (*helveticus* or *vulgaris*) larvae. In fact, intuitively, larval cannibalism will not occur frequently among *Triturus* species because larval size differences do not often allow one larva to fall within the prey size range of a bigger larva and the rate of larva to larva encounter may be quite low in the natural habitat, where *Triturus* larvae do not seem to occur at high densities as do the anura. Harris (1987) postulated that the spatial complexity of natural ponds prevents cannibalism that is otherwise observed in *Notophthalmus viridescens* larvae in laboratory experiments.

However, it should be noted that the effectiveness of analyses of stomach contents may be in some doubt. Hutcherson et al. (1989) observed cannibalism among larval *A. annulatum*, but did not find any larval remains in stomach analyses. It would therefore seem likely that either larval body tissues are rapidly digested or stomach analysis is not sensitive enough to detect low rates of cannibalism among urodeles.

To investigate the likelihood and significance of larval cannibalism and inter-specific larval predation it is necessary to:

i) Establish the size difference between predator and prey.

ii) Establish how frequently this difference will occur in a wild population.

iii) Consider a rate of larva to larva encounters in the natural situation.

Kusano et al. (1985) have examined larval cannibalism in *Hynobius nebulosus*. Cannibalism occurred in laboratory studies with 40-60 larvae per 1.5 litres of water (larval densities in a pond at hatching were 400-600 per m^2). The size ratio of victim to cannibal in this species was recorded as being between 0.4 and 0.8 to 1 and Kusano (1981) points out that overwintering larvae of this species are important predators of the newly hatched larvae.

Genetic Inheritance Genetic effects on larval growth parameters have already been discussed in Chapter 4. (eg. Travis 1981). Examination of paternity effects on larval growth in *Triturus* may be possible, but not easy. It would entail *in vitro* fertilization of

ova. However, the prolonged oviposition period of *Triturus* species would limit the number of ripe ova available at the time of sacrificing a female, and this constraint may will render this approach impractical.

Size difference competition Woodward (1987) in a laboratory-rearing study of the larvae of *Bufo woodhousei* recorded slower growth and lower survival among larvae when reared with a bigger conspecific. This effect was not evident when similar sized larvae were grown together. *B. woodhousei* is a prolonged breeder. This type of inhibition may also occur in urodeles through aggressive interactions (Smith, 1990).

Interactions with other species The presence of animal species other than prey and conspecifics undoubtedly has effects on their behaviour and growth of amphibian larvae. Amphibian larvae may decrease activity levels (Lawler, 1989) or shift microhabitat as a response to the presence of predators such as fish (Semlitsch 1987b) and adult newts (Morin 1986) . Both responses may have consequences for growth. A change in microhabitat may force larvae into less favourable growth environments with a subsequent decrease in growth rate. This may be true in the study of Semlitsch (1987b) which showed that the presence of a fish predator caused reduced larval body sizes in *Ambystoma maculatum* and *A. talpoideum.* Alternatively this effect may have been due to increased competition with the fish for prey items.

Competitive interactions can occur between species from different taxonomic groups. Morin et al. (1988) allowed insects to colonize some of their cattle watering trough 'ponds' and found that insect larvae competed with larval *Bufo woodhousei fowleri* and *Hyla andersonii* by reducing the amount of periphyton available for grazing. More unexpectedly, Alford (1989), using 1.5 m x 60 cm deep cattle troughs in a multi-specific study, found that the larvae of *Bufo americanus* and *Hyla chrysoscelis* seemed to inhibit the growth of larval *Notophthalmus viridescens.* Since newt larvae are planktonivorous and the anuran larvae in question are grazers of periphyton, the mechanism of this effect is

not clear. Such intertaxonomic interactions may form an important aspect of the ecology of *Triturus* larvae, which can occur at relatively low densities, but in lenthic environments where the density of other predatory species can be very high.

Chapter 6
Comparison of Larval Growth Between Two Populations of *Triturus vulgaris*

Introduction Body size of adult smooth newts can vary between populations (see Table 6.1), even when the populations occur within the same locality (eg. Bell 1966 and Beebee 1983). Since female body size is correlated with fecundity (Chapter 3), individuals from a population with a small mean adult body size would be expected to have reduced fecundity. So why does this apparently maladaptive condition exist? There are three possible explanations which are outlined below:

1. Adult populations may have different age structures. In species with indeterminate growth, younger populations would consist of smaller individuals.
2. Environmental constraints on growth and maturation. Small adult females could occur as a result of growth responses to adverse biotic or abiotic environmental conditions.
3. Evolutionary divergences and adaptations in life history strategies. Different habitats select for different life history characters. For example a habitat that exerts high juvenile mortality may select for attainment of sexual maturity at an earlier age than in a population from a low mortality environment. Different environments may select for different life history characters, which may be associated with interpopulation body size differences.

Bell (1977) attributed body size differences in smooth newts within one locality to different age structures of the populations. ie. a population of small animals would be one consisting mainly of young individuals. However more recently it has been shown that ageing newts by body size is unreliable (see Chapter 1). Old populations could well be the ones consisting of larger animals, but more rigorous testing of the hypothesis is needed. Beebee (1983) recorded that along a 10 km transect, body size of smooth newts varied in a

manner related to the habitat type. Newts on heathland ponds were much smaller than newts in farmland ponds (see table 5.1). Beebee rejects differential age-structures as an explanation of size differences between populations in the transect, since there was no overlap in body size between heath and non-heath newts.

As alternative 2 (above), differences in body size may simply reflect the effect of different environments acting on the growth of newts. Beebee suggests this as a possible cause for the reduced body size of his heathland adults. This is the most parsimonious explanation since heathland is typically a nutrient-poor environment.

However, small body size can also be associated with earlier maturity (eg. *Rana sylvatica*, Berven (1982) and *Desmognathus ochrophaeus*, Tilley 1980). Hence in populations where juvenile mortality is high, selection may act to reduce the age of maturity. The loss in fecundity associated with the corresponding decrease in body size is theoretically outweighed by the high mortality risk incurred with delaying maturity. Alternatively, Berven (1982) proposed that environmental factors acting on larval growth exert selection on other life history parameters. In high altitude populations of wood frogs (*Rana sylvatica*) where the growing season is short, selection acts to reduce larval period and yet maximizes size at metamorphosis. Large female frogs produce bigger eggs and these eggs give rise to faster growing larvae. Berven's argument is that larger female body size is selected to produce these large eggs and hence the mountain populations mature later and at a larger size than the lowland populations. Note that this is similar to the first hypothesis in the respect that the adult populations would both have different age frequency distributions. However they are different in that the body size difference is explained in the former hypothesis by differing proportions of individuals from the same range of adult ages, whereas the latter necessitates that one population matures before the other.

It can be difficult to separate out hypotheses 2 and 3. If two populations of the same species, occupying markedly different habitats, have different life history patterns then both explanations may appear to be equally applicable. For example, Tilley (1980) studying *Desmognathus ochrophaeus* at two different elevations, found that the lower elevation

population had smaller size at maturity and matured a year earlier than the high elevation population. However, it is impossible to determine whether these life history differences are due to different selection pressures due to higher predation and/or competition at the lowland site (due to the presence of *D. monticola*), or because of environmental differences between the sites producing different growth responses in the salamanders.

To separate out hypotheses 2 and 3, for smooth newts, larval growth of two populations, of differing body size, was examined. The populations selected were from Milton Keynes, where adults are large and Woolmer Forest, Hampshire, the site of Beebee's survey, where they are small. In the present study a 'common garden' approach was adopted, in that larvae were grown under identical conditions in the laboratory in order to record larval growth. The larval stage encompasses the period of fastest pre-maturity growth in *T. vulgaris* (see Chapter 1) and so any differences in growth rate should rapidly become apparent. If larval growth between the two samples is similar then it would suggest that the adult size difference is a reflection of environmental effects on growth during the pre-maturity stages. However, if the local larvae grew to become larger metamorphs it would suggest that there are genotypic differences between the populations, controlling growth.

When evaluating differences in reproductive success between populations of differing mean body size, it may be important to consider lifetime reproductive success. Within a population small females have lower annual fecundity, and assuming survival is not size-related then they will also have lower life-time fecundity. However, body size differences between populations may also be accompanied by life-history differences, with possible effects on life-time reproductive success. For example in Berven's (1982b) study of *Rana sylvatica*, lowland females were smaller than mountain females because they matured earlier, and lowland females consequently produced smaller clutch sizes. However, since they also start breeding earlier, it is unwise to draw the conclusion that they have lower lifetime reproductive success than the larger mountain females. Hence, a population of small-sized amphibians may not necessarily consist of females with lower lifetime reproductive success than females from populations with a larger mean body size.

	Male			Female			
	TL	SVL	n	TL	SVL	n	Location
Baker (unpubl. 1987)	86.1	44.6	27	83.5	44.9	32	Linford
Baker (unpubl. 1988)	94.0	47.3	18	90.5	47.8	15	Stony Stratford
Beebee (1983)	75.6	-	5	67.8	-	9	Hampshire (heath)
Beebee (1983)	85.5	-	8	83.1	-	8	Hampshire (farmland)
Bell (1966)	78.9	-	131	75.9	-	131	Leicestershire
Clifford (1986)	-	-	-	78.3	42.0	112	Lincolnshire
Halliday (unpubl. 1976)	91.5	46.3	68	86.0	45.5	23	Bromhall, Oxford
Halliday (unpubl. 1976)	84.5	43.5	32	82.1	43.4	8	Hill End Camp, Oxford
Halliday (unpubl. 1976)	79.6	40.9	14	76.9	40.8	11	Kidlington, Oxford
Harrison, Gittins and Slater (1984)	82.1	-	125	79.8	-	266	Mid-Wales

Table 6.1 Body sizes of *Triturus vulgaris vulgaris* from different localities in Great Britain. Total length (TL) and snout to vent length (SVL) are given in mm.

Methods

Adult body size Adult newts were collected in Milton Keynes, by hand, as they migrated towards two ponds (Great Linford and the Open University campus) in the spring of 1988. The females were used in the body-size ovum-size investigation in Chapter 3. For description of maintenance see Chapter 3. The Woolmer Forest newts were captured by net, from a ditch on the edge of the heath, on 11-5-88. The ditch is situated on the edge of an area of lowland heath. The predominant surrounding vegetation is *Calluna vulgaris*, *Erica tetralix*, *E. cinerea* and invading pine and birch. See Beebee (1983) for full description of site. These females were split into two groups and each group was maintained in a glass aquarium as described in Chapter 3. with artificial 'weed' to allow females to oviposit. Seven newts were maintained in a room maintained at 12^{o}C with a photoperiod of 16:8 L:D and a dusk period whilst the other eight were placed in a tank in the east facing window of an unheated room, exposed to natural photoperiod and twilight. The newts exposed to temperature fluctuations and natural photoperiod produced more ova and had a longer period of oviposition than the newts maintained under more artificial conditions. After oviposition, all newts were anaesthetized and measured as described in Chapter 3.

Ova were collected and measured as in Section. 3.1 to establish whether there were egg size differences between the two populations.

Rearing larvae Larvae were reared individually as described in Section 4.2. A rearing temperature of 25^{o}C was chosen to accelerate the larval growth period and so minimize the running time of the practical work. Note that the Milton Keynes larvae are the same sample that was used in Section. 5.1, to investigate the effect of temperature on larval growth. The following measurements were taken during larval growth:

Size (TL) at Gallien and Bidaud stage 40.

Size (TL) at transformation.

Length of larval period.

Results

Adult body size Mean adult body sizes (SVL in mm) for each sex from the two different populations are given below. In addition, body sizes of newts collected by Verrell (Verrell and Francillon 1986) are given for comparison and future reference.

	Male				Female			
Source	SVL	S.D.	range	n	SVL	S.D.	range	n
Milton Keynes	46.5	2.5	39.0-53.0	58	45.4	3.0	39.0-52.0	53
Woolmer Forest	42.1	2.0	37.5-46.0	17	40.3	2.1	37.0-45.0	15
Verrell and Francillon (1986)	45.5	2.9	-	26	45.1	4.1	-	24

Table 6.2. To show SVL of adult newts from the Woolmer and Milton Keynes populations and Verrell and Francillon's study (1986). Verrell collected newts from Great Linford and Soulbury (Buckinghamshire), Marston (Oxfordshire) and Littleworth Common (Hertfordshire).

The newts captured at Woolmer were in fact larger than those described by Beebee (1983) (mean TL 83.9 and 75.8 for males and females respectively), falling between the sizes he recorded for heathland and farm land. This may be because the newts from the present study were collected from the heathland edge rather than further onto the heath. Hence there is some overlap in body size between the two populations. However, the Woolmer females were still significantly smaller than the Milton Keynes females (mean SVLs 40.3 mm and 45.4 mm respectively, $t = 6.24$, 66 d.f., $p < 0.001$).

In order to show that size differences between adults of the two study populations were due to pre-adult growth it is necessary to demonstrate that age structure differences could not have produced the size difference. It is possible that the Woolmer animals may be exposed to high adult mortality and so consist of first time breeders only. Verrell and Francillon (1986) show that males probably breed for the first time at two years, whilst

most females are a year older. Using equations derived from linear regressions of SVL on age from their original data it is possible to calculate body size at sexual maturity for smooth newts in Verrell and Francillon's sample. It is argued that the size-age structure of my Milton Keynes sample will be similar to that of Verrell and Francillon. 32 of their newts came from the same study site and the mean SVL measurements of the two samples are similar. T-tests on the SVL of males and females from Verrell and Francillon's and my own data show that these two samples are statistically unlikely to have come from different size populations. For males, $t = 1.632$, 82 d.f., $p = 0.103$ and for females, $t = 0.475$, 75 d.f., $p = 0.641$. So for the purposes of this study, Verrell and Francillon's data will be used to generate information which will be pertinent to my own sample. Regression of SVL on age for the two sexes, in Verrell and Francillon's sample gives the following equations:

Males:	$y = 1.339x + 40.827$	$r = 0.53$	$p = 0.006$
Females	$y = 1.393x + 40.093$	$r = 0.298$	$p = 0.154$

Assuming that the minimum age of sexual maturity is two years for males and three for females, and substituting 2 and 3 for the *x* value in the above equations, then SVL at maturity is calculated as 43.5 mm for males and 44.3 mm for females. So, even if Woolmer newts were all first year breeders, their mean SVL is still smaller than first year breeders in the Milton Keynes population. However, these two calculated values do fall within the 95% confidence intervals for the SVLs of the Woolmer populations (38.1 - 46.1 mm for males and 36.3 - 44.3 mm for females. This means that differential population age structures could account for the body size differences between the two populations.

Ovum sizes Woolmer ova (mean diameter = 1.31 mm) were significantly smaller than ova produced by females collected in Milton Keynes (mean diameter = 1.35 mm). See Table 6.3.

Larval growth Despite the difference in ovum sizes, there was no significant difference between size at hatching, larval period or size at transformation. See Table 6.3. Growth rate was calculated as increase in TL per day, and size-specific growth rate was calculated as size increase per day, relative to initial size, calculated as increase in TL per day multiplied by the reciprocal of TL at stage 40. Woolmer larvae exhibited faster absolute and specific growth rates, because the Woolmer larvae tended to be slightly smaller at stage 40, but grew to a slightly larger size at transformation and take a little less time attaining the latter stage. See Table 6.3.

	Milton Keynes			Woolmer				
	Mean	s.d.	n	Mean	s.d.	n	t	p
Ovum diameter	1.35	0.480	108	1.31	0.329	72	2.85	0.005
TL at stage 40	9.22	0.281	13	9.09	0.266	19	1.17	0.251
TL at transformation	36.5	4.366	13	37.1	3.839	19	1.63	0.113
Larval Period	39.6	4.788	13	36.9	7.534	19	1.15	0.259
Growth rate	0.689	0.092	13	0.769	0.070	19	2.81	0.009
Specific growth rate	0.075	0.008	13	0.085	0.008	19	3.56	0.001

Table 6.3. Ovum sizes and larval growth parameters of Milton Keynes and Woolmer samples. All size measurements are given in mm, and larval period in days. T-values and probability levels are given to compare each parameter between the two populations.

Discussion A comparison of data on body sizes of smooth newts from Woolmer Forest with data from newts collected in Milton Keynes by myself and Verrell and Francillon (1986) does not rule out the hypothesis that body size differences between these two populations are due to differential mortality. It is possible that a high rate of mortality at the Woolmer site reduces the mean age of the adult population, with the result that the newts at this site tend to be small because they are relatively young adults. Smooth newts can be aged by skeletochronology, using the methods described in Verrell and Francillon (1986). However a requisite of this technique is that the newts are killed. Newts were collected at Woolmer on the understanding that they would be returned to the site, alive.

From the data that has been obtained, it is demonstrable that the Woolmer population of smaller individuals produces smaller ova. This may be a body size effect since an investigation of the relationship between body size and ovum size within the Milton Keynes population (Chapter 3.) reveals that mean ovum diameter is positively related to female body size. However, it is impossible to be certain that this egg size difference is a function of body size, or whether it is an effect of different feeding environments on the females, or whether it has been naturally selected due to different environmental demands. Petranka et al. (1987), examining populations of *Ambystoma texanum* from differing environments, found that the pond dwellers (smaller females) produced smaller ova than the stream dwellers, but no body size-ovum size correlation was actually found within populations. The study concluded that large ovum size was an adaptation to the stream environment rather than a body size effect. Hence the results obtained in the present study could reflect a genetic response to two different environments. However, this possibility is not predicted by some life history theory, which notes that larger ova produce faster growing or better competing larvae, and thus predicts that larger ovum size is selected by harsh growth environments (Sibly and Calow 1986). Although there is not enough quantitative information in the present study to reveal the effects that the differing environments have on the reproductive strategies of these two populations, simple observation suggests that the Woolmer population is situated in a harsher growing environment. The heathland pools

and ditches appear to have a low prey biomass and are subject to desiccation during the summer months. The terrestrial environment may also present newts with low rates of resource accrual, possibly affecting adult body size. However, the results of the present study show that the heathland population produced smaller, rather than larger ova.

This leaves the third alternative that reduced feeding opportunities in the terrestrial environment may subject individuals to low rates of resource accrual, possibly affecting the resources available to females for oocyte production and resulting in small ova. Hence female smooth newts living in a food impoverished environment may not be able to acquire enough resources to produce ova as large as current life history theory predicts.

Although the Woolmer females tended to produce smaller ova, this seemed to have little effect on size at hatching, since both samples of larvae produced similar sized hatchlings. Subsequent growth actually showed the opposite trend to that predicted by the general finding that within a species, smaller ova produce slower growing larvae (Section 2.1), since the Woolmer larvae tended to grow faster than the Milton Keynes larvae. The negative egg size effect on larval growth is, however, consistent with the finding of Section 5.4, that in the laboratory, no advantages of large hatchling (and by inference large ova) are detectable.

The faster growth of the Woolmer larvae could be due to genotypic selection for fast larval growth. However, the fact that under laboratory conditions, they were able to grow as large as larvae from the Milton Keynes population is suggestive that small body size of the adult population of Woolmer newts may be partly due to environmental effects acting on growth. This assumes that growth to maturity is strongly affected by growth responses generated during the larval stage. There is evidence that this is true within populations (see Section. 4.1).

This comparison of larval growth between two populations is confounded by the ovum size difference between the populations. Woolmer females produced smaller ova. The predicted effect of reduced ovum size on larval growth is that larval growth rate should also be reduced (see Sections 3.1 and 5.4). Clearly this was not the case in the present

study, so the proposition that there may be a genetic component, that produces faster growth in the Woolmer larvae is still valid. However, to fully disentangle the genotypic and environmental factors influencing growth, using a 'common garden' rearing technique, it would be necessary to rear larvae to maturity and measure their ovum sizes, to establish whether or not the ovum size difference has a genetic basis. If the ovum size difference between the two populations disappeared, then it would allow control of ovum size if subsequent larvae were reared.

Tilley (1973) surmized that variation in adult body size between localities could be potentially due to three possible scenarios:

1. Constant age at maturity but variable growth rates.
2. Constant juvenile growth rate but variable age at maturity.
3. Variable growth rates and variable age at maturity.

Different urodele species may fall into different categories. For example, in his study of *Desmognathus ochrophaeus*, populations at varying altitudes and varying habitat types within a similar altitude, Tilley (1973) concluded that this species fitted the second pattern, displaying similar juvenile growth rates, but varied ages of maturity. He proposes that this is due to differential survival between localities. When there is a high probability of survival, populations delay the onset of maturity to take advantage of increased fecundity due to larger body size. Bruce (1988) used the same logic to explain body size differences among desmognathine species. He proposed that body size differences between the species occur due to different ages of sexual maturity. Bigger species are older at maturity due to a longer period of growth. He also proposes that selection for smaller body size may be due to selection to reduce reproductive age due to competition/predation pressures. He notes that body size reduction is also accompanied by a more terrestrial mode of life. Within *Triturus*, however, I suggest that selection for lower age at first reproduction is more likely to follow a move to terrestrial life. Terrestrial growth conditions may not be as favourable as aquatic conditions, which may select for attainment of maturity at a smaller body size. Hence populations would exhibit variable growth rates as well as variable ages

at maturity, like Healy's (1973) *Notophthalmus viridescens* populations, which had differing growth rates and ages of maturity, dependent on the degree to which they exploited that aquatic environment (although there were no adult body size differences).

Interactions between species have been proposed as causing variation in body size among populations of a single species in urodeles. The smaller of two species may suffer reduced body size due to competition for resources or behavioural avoidance of the larger species, leading to reduced foraging and growth (eg. Davic 1983). However, such biotic constraints are unlikely to be acting in the present situation, since the large body size population of smooth newts is allotopic with a larger congeneric (*T. cristatus*) and the small body size population is allotopic with a smaller species (*T. helveticus*). If interspecific interactions, based on body size differences, did occur, then they would be expected to generate interpopulation body size differences among *T. vulgaris*, in the opposite direction to those observed.

Conclusion Two populations of smooth newts, from differing types of habitat, differed in adult body size. This size difference seems unlikely to be due to genotypic control, since under a 'common garden' rearing study, there was no difference in body size at transformation. It is therefore likely that this size difference is either a function of age or differing growth environments in the natural situation.

Chapter 7
Effect of Timing of Oviposition on Larval Growth

Introduction This rearing trial was carried out to answer the specific question of how timing of oviposition affects the growth of newt larvae. As described for smooth newts in Section 3.1, female newts in the *Triturus* genus oviposit large clutches (88-637 eggs per female smooth newt, see Section 3.1) by secretion of individual ova in folded plant leaves or other flexible matter. This individual egg production protracts the oviposition period so that as much as three months may elapse between the first and last ova being produced within any particular season (see Oviposition Time Course in Section 3.1). This variation in timing of individual ovum production seems likely to affect the growth of the subsequent larvae, since such factors as water temperature, prey availability and larval density will alter as the season progresses. All three of these factors are potentially able to affect larval growth (Chapter 5).

Accounts of the effect of timing of oviposition on urodele larval growth are given by Bell and Lawton (1975), Bell (1977) and Harris (1980). Bell and Lawton (1975) concluded that *Triturus vulgaris* larvae grew in three age cohorts, generated by three peaks in female oviposition, and Bell's (1977) work suggests that the last cohort may not experience a long enough growing season to allow it to transform before the winter. Harris (1980) describes a population of *Ambystoma maculatum* that bred in a temporary pond, in three waves spread over two months. The earliest wave was exposed to a period of freezing, which resulted in very high egg mortality. However, it is not clear whether direct freezing or low levels of dissolved oxygen killed these ova. Morin et al. (1990) found that in the prolonged-breeding treefrog, *Hyla andersonii*, larvae spawned later in the season suffered from decreased growth and survival. So, it would appear that timing of oviposition may well affect the success of larvae within a prolonged reproductive period.

The present study was designed to investigate the effect of timing of oviposition on larval growth and body size at transformation. The study compared the growth of larvae

from ova oviposited early in the season with larvae from ova oviposited late in the season.

Growing larvae in the laboratory was adopted as the main technique of investigation of larval growth within the present project, to provide a standardized growth environment to allow the manipulation of one variable at a time and because newt larvae in the field cannot be aged. However such a controlled rearing regime would not allow any meaningful effects of the timing of oviposition on larval growth to be recorded, since seasonal variation in growth conditions has been removed. Hence, the present study adopted the use of artificial ponds in a similar approach to that of Wilbur and Morin (eg. Morin 1983, Wilbur 1987). They have used cattle watering troughs in a series of experiments to provide more or less identical ponds in which they could manipulate environmental components (eg. species composition, densities of larvae and predators) with the additional advantages of facilitating easy sampling and allowing rigorous experimental design and statistical analysis.

This approach does have its limitations and critics. Petranka and Sih (1986) draw attention to the fact that artificial ponds (cattle troughs) may contain artificial prey community compositions or depressed levels of prey abundances. Both of these factors will affect larval growth and perhaps over-emphasize the effects of larval density, although Wilbur (1989) and Morin (1989) both claim that their densities fall within the range exhibited in natural ponds. Jaeger and Walls (1989) pointed out that uniform tank depth does not replicate the full range of microhabitats exhibited by a natural pond, that initial larval and predator densities may exceed natural populations and that the netting lids used in some experiments exclude potential insect predators and prey.

Despite criticism, artificial ponds must be seen as the closest it is possible to be to a truly natural situation, except perhaps for the use of enclosures, containing larvae within natural ponds. Field enclosures were not adopted in the present study, because their use requires either the division of a large number of natural ponds, which were not available, or the construction of many enclosures within a single pond. The latter possibility suffers from the drawback that such enclosures can artificially increase larval survivorship

(Petranka and Sih [1986] point out that field enclosures may raise larval densities by as much as 10-15 times) and hence still do not achieve the aim of growing larvae in a natural situation. In addition, a requisite of the present study was to monitor the course of larval growth, which seemed to be easier to achieve using artificial ponds rather than enclosures.

Triturus cristatus was selected as the study species since its relatively large ova are easily detectable in natural ponds and its large larvae would be easy to recapture in the artificial ponds during the larval growth period, allowing body size measurements to be made.

Methods It was decided to compare the growth of the first larvae to hatch in the season with the larvae that hatched at the late end of the hatching period. The two groups of larvae are referred to as 'early' and 'late'. Larvae were reared in 16 artificial 'ponds' laid out in a 2 x 8 randomized block design in order to counteract any positional effects. Assignment of tanks to early or late larvae, within each block, was decided by coin toss. 16 glass aquaria 120 x 37.5 x 37.5 cm were used as the artificial ponds. It was intended that these aquaria should replicate natural pond conditions as closely as possible. So, each aquarium was sunk into the ground to a depth of 27 cm and a mixture of soil and sand back-filled around the sides to provide a large surface area of soil in contact with the aquaria so that the ground buffered the ponds against large temperature fluctuations (see fig. 7.1). Each tank was filled to a depth of 27 cm with unconditioned tap water, so that each held 121.5 litres. After two days each tank was seeded with *Daphnia* from an already established culture. One week later the first cohort of newt larvae was added.

T. cristatus ova were collected from a deep, permanent pond on the campus of the Open University, Milton Keynes, England. Water temperature was recorded, on days when ova were collected, in the weed bed used by the newts to oviposit. Ova were collected, under licence from the Nature Conservancy Council, by unwrapping them from the leaves of *Myosotis palustris*. These ova were then kept in the individual cells of tissue culture dishes, which were wrapped in green polythene to exclude direct sunlight, and returned to the pond, so that the ova would hatch under natural conditions of temperature and light and develop to Gallien and Bidaud stage 40, whereupon they were transferred to the field tanks. Two cohorts of eggs were taken, one from the beginning of the oviposition period, and one from the end of the oviposition period. The first ova were detected in the pond on 21-3-89 and ova for the last cohort were collected on 30-5-89, suggesting a population oviposition period of 70 days. In actual fact oviposition probably lasted for longer than this, since 30-5-89 was the last day on which a sizeable cohort of freshly produced ova could be collected; smaller numbers of eggs were very probably oviposited for some time after this date. Most of the early cohort reached Gallien and Bidaud stage 40

on 14-5-89. 35 larvae at this stage were measured with an eyepiece graticule and binocular microscope (TL in mm) and were introduced to the field tanks (five larvae per tank). The second cohort attained stage 40 on 7-6-89, 24 days later, and was treated similarly.

Early larvae were measured on days 24, 36, 48, 60, 72, 84 and 96 of larval life, whilst late larvae were measured on days 12, 24, 48 and 72. TL was measured to the nearest 0.5 mm, using the graduated trough described in Section 4.2.

To prevent efts escaping after transformation, mesh lids (3 mm guage) were placed over each tank just as larvae neared the end of the larval stage. Leaving the tanks uncovered until this last stage had the advantage of allowing insects to colonize the ponds, with the consequence that larval mosquitos and chironomids were seen in all the tanks, providing food for the newt larvae. No predatory insects colonized, despite the close proximity of the parental breeding pond (300 m) which contained large numbers of dytiscid beetles.

At transformation, larvae were anaesthetized and TL and SVL were measured to the nearest 0.5 mm as described in Section 4.2. On recovery from anaesthesia all larvae were released at the pond of origin.

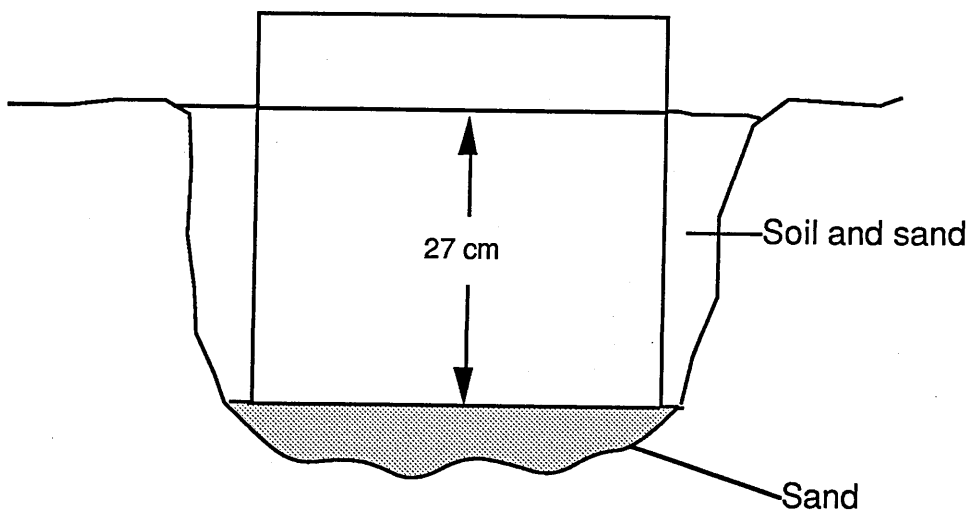


Fig 7.1. Cross section of aquarium sunk into ground, to create artificial pond.

Results Growth curves of the two larval cohorts are shown in Fig 7.2(a). As can be seen from the graph, the late cohort exhibited faster growth than the early larvae. It was initially intended to use an ANOVA to analyse the data generated from this study so that variation due to tank effects could be calculated. However, the high larval mortality, reducing numbers of larvae to between zero and three per tank, meant that a t-test was more appropriate to compare parameters of the early larvae with those of the late larvae.

The metamorphs from both cohorts were of a similar size. SVL of early cohort was 45.4 mm and that of the late cohort was 44.9 mm ($t = 0.585$, $p = 0.565$). However, the early cohort had a longer larval period of 90.8 days whilst that of the late cohort was 75.1 days ($t = 7.006$, $p < 0.001$). Hence rate of differentiation was faster in the late cohort. Larval growth rate was calculated as change in TL per day of larval growth, as described in the Methods section of Section 5.1. The late cohort grew significantly faster than the early cohort (0.91 mm/day and 0.77 mm/day, $t = 5.684$, $p < 0.001$). See Table 7.1 for summary of these results.

It can also be seen from graph in Fig 7.2(a) that the variation in individual larval periods caused an overlap in the timing of transformation between the two cohorts.

	Early			Late				
	Mean	s.d.	n	Mean	s.d.	n	t-value	p
SVL	45.4	2.66	12	44.9	1.19	11	0.585	0.565
Larval period	90.8	4.63	12	75.1	6.11	11	7.006	<0.001
Growth rate	0.77	0.04	12	0.91	0.08	11	5.684	<0.001

Table 7.1. Mean SVL (mm), larval period (days) and growth rates (mm/day) of early and late *T. cristatus* metamorphs from artificial ponds.

Comparison with metamorphs from laboratory and a natural pond The metamorphs from this study were large in comparison to sizes reported elsewhere. To demonstrate this large body size, size of metamorphs is compared with sizes of metamorphs from a laboratory rearing study and from a natural, local pond. Mean snout-vent lengths of the three groups are given in Table 7.2.

Artificial pond metamorphs were significantly larger than those from reared in the laboratory ($t = 4.662$, $p < 0.000$). The laboratory metamorphs were in turn larger than those from the natural pond ($t = 3.027$, $p = 0.004$). Metamorphs from the natural population also appear to have poorer weight conditions than either the metamorphs from the artificial ponds or the laboratory study (see regression lines in graph in Fig. 7.2b)..

To examine whether metamorph body size increased with increasing larval periods, Pearson product-moment correlations were performed on SVL at transformation and larval period. There was a significant, positive correlation for the early cohort, $r = 0.77$, $n = 12$, $p < 0.01$, but a non-significantly positive correlation for the late cohort, $r = 0.34$, $n = 11$, $p > 0.05$.

		mean	s.d.	n
SVL	Artificial ponds	45.1	2.064	23
	Laboratory Study	42.2	1.918	18
	Natural pond	39.6	3.285	27

Table 7.2. Mean SVL (mm) of *T. cristatus* metamorphs from artificial ponds, natural ponds and the laboratory.

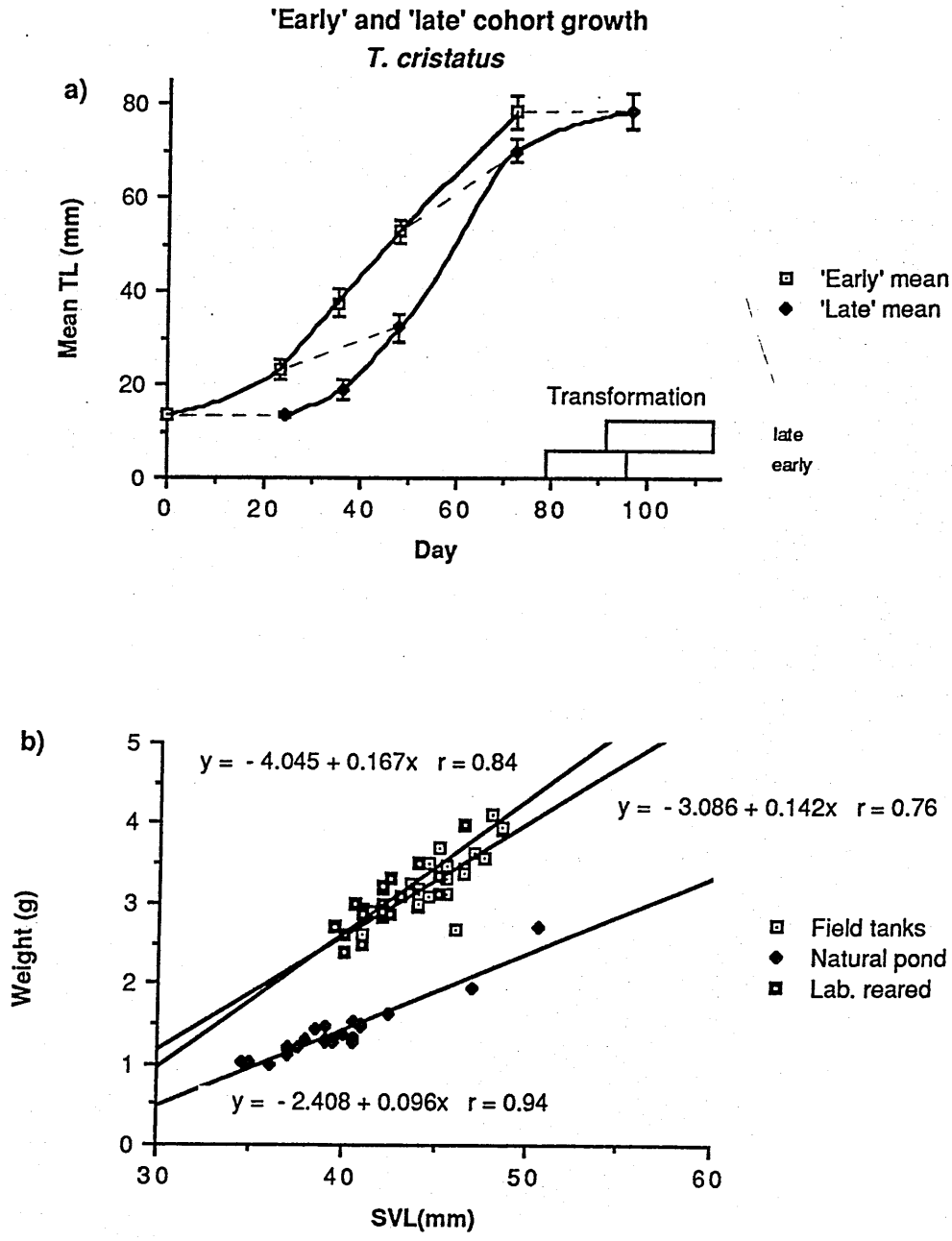


Fig. 7.2. Graphs of growth of *T. cristatus* early and late cohorts and weight condition of metamorphs. (a) Growth curves for both cohorts. Broken lines represent measurements made at comparable times during each larval period (days 0, 24, 48 and 72). (b) Metamorph weight plotted against SVL, to show weight condition for metamorphs from cohorts, a natural pond and the laboratory.

Discussion The prolonged oviposition period of the study population, in excess of 70 days, is typical of *Triturus* species (see Section 3.1). The specific aim of the present study was to examine the effect of differential timing of oviposition, and hence hatching, on the subsequent growth of larvae. However, it is noticeable that the range of oviposition dates is larger than the range of hatching dates. A 70 day range of oviposition was reduced to a 24 day difference in time to attain Gallien and Bidaud stage 40. This effect was most probably due to the gradual, seasonal temperature increase. At the beginning of the oviposition period, pond temperatures in the areas selected for oviposition were 9-11^{o}C, whereas by the end of this period temperatures had risen to 15-20^{o}C. So it would seem that ova produced later in the year hatch faster than early ova and produce larvae that also grow faster. Metamorphs from the two cohorts were similar in size at transformation, so there can be no size-related selection pressure for either early or late oviposition. However there are advantages to both options in terms of growth. The faster developmental time of the late cohort may well be advantageous in a real pond situation because it will allow escape from predation by size and a wider selection of prey items (see Ch 4.1). However there are obvious advantages in early oviposition. Early oviposition may be selected by the threat of pond desiccation, because although the early larvae take longer to attain transformation, they still complete the larval period earlier in the year than late larvae and so would have better chances of escaping a drying pond. Earlier oviposition and completion of the larval phase would also mean that these metamorphs would have a longer growth period in their first year of life and so they may be a larger size than late larvae by the end of the growth season. There may well be a mixed selection pressure on timing of oviposition.

The overlap in timing of transformation of metamorphs from the two cohorts helps to explain the discrepancy between laboratory rearing studies and field observations concerning size and length of larval period. Chapter 4. showed that newt larvae display intrinsically variable larval periods and that longer larval periods were associated with larger size of metamorphs. However, there is no evidence of metamorph body size

increasing with time in the field (Verrell 1985, Harrison 1985). Results of the present study show that a cohort of ova, oviposited at the same time will transform over a long period of time, so that larvae with intrinsically short larval periods (small metamorphs) will transform before larvae from a previous cohort with intrinsically long larval periods (large metamorphs). Hence, on any day of metamorph emergence from a natural pond, there will be a range of metamorph body sizes, that will remain fairly constant for most of the emergence period (see Fig. 7.3).

Comparisons with laboratory growth patterns The faster growth of the late cohort is predictable in the light of the finding that larval growth is sensitive to temperature (see Section 4.2) and that the late cohort would be exposed to warmer seasonal temperatures during its growth period. However, growth of *T. vulgaris* under laboratory conditions also predicts that this late cohort should also produce larger metamorphs. Why this did not occur is not apparent, but it may be that the larvae grown in this study attained an upper size limit, as postulated in the Wilbur-Collins model (1973). Certainly the metamorphs measured in this study are the largest *T. cristatus* metamorphs on record.

The growth curves are of a similar shape to those obtained from *Triturus* larvae reared in the laboratory. The mean larval period of the late cohort was similar to the larval period for those larvae reared at a constant temperature of 20°C in the laboratory, 75.1 and 76.9 days respectively. This is rather surprising in the light of the fact that the experimental ponds must spend a good deal of time at temperatures rather lower than that of the laboratory. From the results obtained from *T. vulgaris* (see Section 5.2) it would seem that development proceeds fastest at higher temperatures. However, the present experiment suggests that constantly high temperatures may not be necessary to achieve an equally fast growth rate. It is relevant at this point to note that trout (*Salmo trutta*)in natural rivers grow faster than laboratory-reared fish on maximum rations (Jensen 1990). Jensen suspected that faster field growth rates may be due to diel temperature fluctuations. Further laboratory experiments employing fluctuating temperatures would be able to establish whether or not this is the case in newt larvae.

Larvae grown in the laboratory showed a distinct increase in metamorph size, with

increasing larval period. However, only the early cohort mimicked this trend. It is suspected that the late cohort did not conform to this pattern, because of the relatively low number of surviving larvae (11 metamorphs) and because the prey populations in the artificial ponds tend to show variation in numbers and species composition with increasing time into the trial. The combination of small sample size and variable growth environments probably served to mask the effect of increasing body size with increasing larval period.

The final difference between the larvae raised in these artificial ponds and those reared in the laboratory is in body size. The metamorphs from the present study were larger than those reared in the laboratory which suggests that some vital component or components of the natural larval environment were absent from the laboratory studies of this project. The situation is complex, however, since the metamorphs from this study were also larger than those from a natural population emerging at the same time. More experiments would shed more light on these size differences, but at present post-hoc explanations will have to suffice.

The findings of this study that timing of oviposition has little effect on metamorph size, but that late ova grow faster and metamorphose later may throw some light on the evolution of the prolonged period of oviposition of single eggs exhibited by *Triturus* species. The move from simultaneous spawning of the whole clutch to deposition of single eggs has also been accompanied by the distinctive oviposition behaviour of *Triturus* which entails the wrapping of individual ova within a leaf which is wrapped around the egg (Smith 1973). It is not possible to pin-point a single factor that led to the evolution of the oviposition strategy exhibited by *Triturus*. Oviposition of single ova may have evolved as a response to the unpredictability of the larval growth environment, as implicit in the above discussion. Alternatively, folding individual ova in plant leaves may have evolved because this serves to protect eggs from predation. It is also possible that oviposition of only a fraction of her total clutch in any single night allows a female to increase total clutch size. If either of the last two hypotheses are true, then the prolonged oviposition period exhibited by newts may be a necessary consequence of production of single ova. Individually

ovipositing and wrapping up several hundred ova takes time, so that there may be no need to invoke an adaptive explanation of timing of oviposition.

Conclusion Ova oviposited early in the year allowed larvae to complete the larval stage earlier. These larvae were of a similar size to the 'late' larvae at transformation, but showed a slower rate of growth and development.

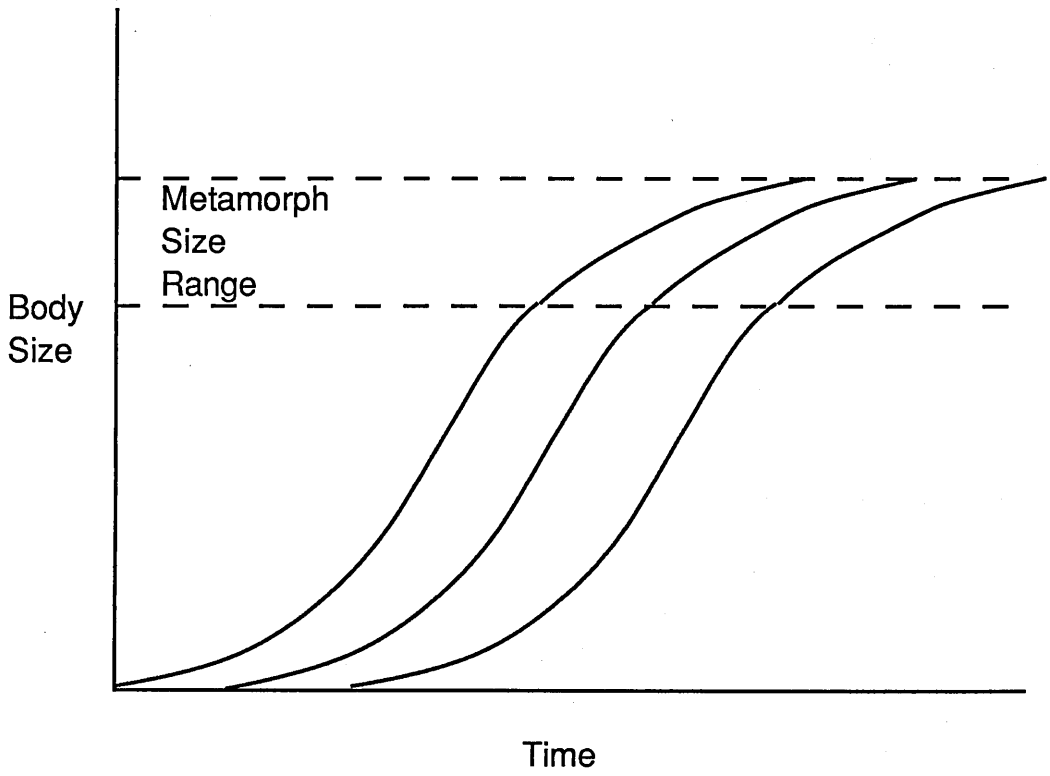


Fig. 7.3. Diagram to show how variable timing of oviposition may mask effect of increasing body size with larval period.

Chapter 8
Growth After Transformation

8.1 General Introduction After completion of the larval phase, newts typically leave the water and spend the next one and a half to four and a half years as terrestrial animals, until attainment of sexual maturity when they return to the water to breed (see Table 8.1). This eft stage may take up a large proportion of the life of a newt and yet there is very little available information concerning the ecology of newts during this period. The lack of data concerning the growth of *Triturus* may be partly due to methodological problems of studying natural eft populations. Newts are difficult to mark, and recapture rate during the terrestrial eft stage, can be very low, due to dispersal. Difficulty in finding juvenile *T. vulgaris* is reported by Dolmen (1983b). Hence most growth data are relevant to adults during the a breeding season (eg. Verrell 1987) or between seasons (eg. Hagström 1974). There are no longitudinal growth data for *Triturus* efts in the natural situation. However, it is known that newts take some time to reach sexual maturity and from this we can deduce that growth during the eft stage is relatively slow. Age at sexual maturity varies with locality and it would be reasonable to assume that this is a reflection of geographic variation in temperature and/or rainfall, and hence the length of activity season of the efts. Some ages at sexual maturity are given in Table 8.1.

Since only about four months of life prior to sexual maturity is spent as a larva, then there is a relatively long period of terrestrial life. Growth conditions during this period may well affect the age and size at first breeding. To circumvent the difficulties associated with studying growth of efts in the field, newts of two species (*T. cristatus* and *T. vulgaris*) were grown under captive conditions, using two different rearing methods.

	Reference	Location	Ageing technique
Triturus cristatus			
3-5	Hagström 1980	Sweden	skeletochronology
4	Steward 1969	General	not given
2-4	Smith 1969	Great Britain	not given
3-5	Dolmen 1982	Central Norway	skeletochronology
2+	Dolmen 1983	S.E. Norway	skeletochronology and
4+	Dolmen 1983	Central Norway	testis lobation
2-3	Francillon-Vieillott et al. 1990	France	skeletochronology
Triturus vulgaris			
3-4	Steward 1969	General	not given
2-4	Smith 1969	Great Britain	not given
2-3	Dolmen 1982	Central Norway	skeletochronology
2+	Dolmen 1983	S.E. Norway	skeletochronology
3+	Dolmen 1983	Central Norway	and
5/6+	Dolmen 1983	Northern limit in Norway	testis lobation

Table 8.1. Ages (years) at sexual maturity for *T. cristatus* and *T. vulgaris*.

Amphibia transform at a wide range of body sizes. Studies of anurans have shown that animals that are relatively large at transformation enjoy higher chances of survival and mature as larger adults (*Rana sylvatica*, Berven and Gill 1983, *Pseudacris triseriata* Smith 1987). Among urodeles, size at transformation has been shown to be related to size at maturity (*Ambystoma talpoideum*, Semlitsch et al. 1988). Hence, the size of amphibia at transformation has been used as a measure of fitness (Wilbur, 1980). If the growth of *Triturus vulgaris* follows the same trend then it can be predicted that larger metamorphs will mature as larger adults than smaller metamorphs. This may be expressed as faster

growth of larger metamorphs. Alternatively, growth rates may be similar, but the larger metamorphs may maintain their size advantage.

There is also some evidence to suggest that larger metamorphs actually grow more slowly than small metamorphs. Healy (1973) found that growth rate of juvenile *Notophthalmus viridescens* tended to decline with increasing body size, and a field study of growth of *Cryptobranchus alleganiensis* (Peterson et al. 1983) showed that growth after transformation declines with body size. If this negatively size-dependent growth occurs immediately after transformation then it is possible that an animal that is small at transformation will grow faster than a larger individual. However, this must be regarded as an unlikely scenario, since smaller metamorphs may not be as physiologically well-equipped to cope with the demands of terrestrial life as well as large metamorphs, as predicted by Pough and Kamel (1984). Data supporting this prediction is provided by John-Alder and Morin (1990). They that small metamorphic *Bufo woodhousei fowleri*, produced by rearing larvae at high densities, had reduced jumping abilities and stamina when compared to larger metamorphs from lower larval rearing densities.

8.2 Growth of *Triturus vulgaris* Efts After Transformation

Introduction The present study looked at growth of individual metamorphs of varying sizes, under constant conditions. The animals used were those from studies of factors affecting larval growth (Chs. 4 and 5), and so conditions experienced during larval growth were not uniform between larvae. The study examined the relationship between size at transformation and growth immediately after this event.

Method Eighty metamorphs were obtained from a laboratory rearing experiment in which larvae were subjected to different levels of feeding and density. At transformation, individuals were anaesthetized in MS-222(Sandoz) in order to obtain an accurate body size measurement. Snout-vent length was measured to 0.25 mm.

Efts were housed in plastic containers (28 x 15 x 10 cm), ten animals per container. Each container was lined with a sheet of tissue paper with another sheet of crumpled paper placed on top of this to provide cover for the efts. The paper was kept moist to provide humidity, and changed once a week. The plastic containers were kept in a controlled temperature cabinet at 22 +/- 1°C. Since photoperiod may affect the growth of post-metamorphic amphibians (Richards and Lehman, 1980), a constant 16:8 L:D schedule was maintained. Boxes were rotated on a weekly basis to avoid any positional effects. Food, consisting entirely of *Drosophila* larvae and adults were provided on an *ad libitum* basis. *Drosophila* was cultured in tubes (8 x 3.5 cm). Once large numbers of larvae could be seen in the food medium, the flies were removed and the tubes placed in the container with the newts. The efts were then able to feed on larvae as they crawled out of the food medium. Individuals were also observed capturing the adult flies.

Efts were grown under these conditions for ten weeks. Each week the animals were anaesthetized in MS-222 and measured.

Marking the efts to allow individual recognition proved to be a problem. Initially a panjet was used to mark the tails or feet with alcian blue (1%). However, this proved to

cause damage to the efts to the extent that feet and tails were severed in some cases. In addition the panjet is not easy to aim with the precision required to mark such a small animal. As an alternative, animals were tattooed with individual patterns of dots on the tail. Efts were tattooed whilst under anaesthetic. To do this a sterilized insect setting pin was pushed through the tail, and the tail then immersed in (1%) alcian blue. To allow ease of recognition, the tattoo was placed on the lower edge of the tail and behind the extended hind foot. This ensured that the foot did not obscure a tattoo of an anaesthetized animal. Marks any higher than the lower edge of the tail were prone to be obscured by pigmentation that developed with age. The tatoos needed to be renewed, usually after two weeks and then, again for some individuals in succeeding weeks. The need for this renewal of tattoos means that, although this technique of marking is suitable for laboratory populations, it could not be reliably extended to the field.

Results Survival of efts under these conditions was low. Only in three out of the eight boxes did any of the efts survive for the ten weeks of the study. Of these, one eft died in two of the boxes, so that the data analysed were obtained from 28 individuals.

Over the seventy days mean SVL changed from 17.8 mm (s.d. = 3.025) to 21.7 mm (s.d. = 3.856), so that the mean change in SVL was 3.9 mm, which represents an increase of 21.6% in SVL.

Growth data of individual efts is presented as a Walford plot (Walford, 1946), (see Fig. 8.1.) which plots body size at the end of the study against body size at the start of the study. From this plot it appears that body size relative to the mean body size of the metamorphic population was preserved over the ten weeks of the study. Efts that transformed at a relatively large body size remained large compared to efts of a similar age. A Pearson product-moment correlation of SVL at transformation and SVL after seventy days of eft life confirms this: $r = 0.96$, $p < 0.01$, 26 d.f.

The graph also shows that the larger metamorphs seemed to increase in SVL more than the smaller efts. For the correlation between SVL at transformation and change in

SVL, r = 0.51, p < 0.01, 26 d.f. However, there was no effect of SVL at transformation on growth expressed as a percentage of SVL at transformation (r = 0.012, p > 0.05, 26 d.f.). Hence, although larger metamorphs tended to grow more in terms of absolute growth than small metamorphs, they grew a similar amount in terms of growth relative to initial body size. The uniformity of relative growth over a wide range of metamorph sizes ensured that there was no increase in size variation over the growth period. The initial coefficient of variation of metamorphs was 16.99 and seventy days later this was 17.81.

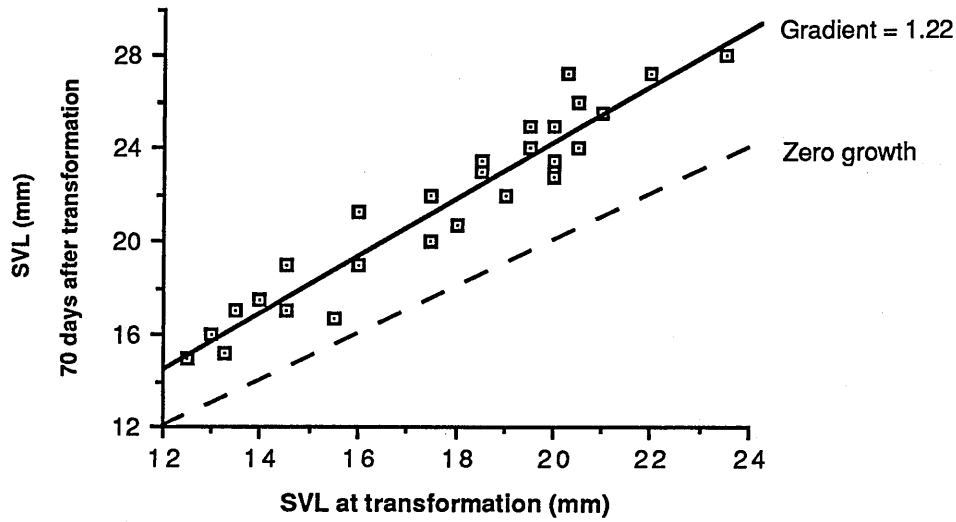


Fig. 8.1. Walford plot to show growth of 28 *T. vulgaris* efts immediately post-transformation. SVL at 70 days post-transformation is plotted against SVL at transformation. A linear regression line has been plotted through the data points and the line of zero growth has been drawn to represent no change in SVL over the seventy day period of growth.

The regression line of SVL after seventy days on SVL at transformation has been added to the Walford plot. The gradient of this regression line is +1.22, which is significantly different from a gradient of 1 (t = 3.02 > 2.78, at 1% level, 26 d.f.). A gradient of one would be expected if absolute growth bore no relationship to body size at transformation. Since the gradient calculated from the data is greater than one, this

confirms that larger metamorphs grow more than small metamorphs.

Discussion Body size at transformation was a good predictor of body size ten weeks after transformation. Although larger metamorphs grew more than small metamorphs, growth relative to body size was similar over the whole range of initial sizes. Hence the relative sizes of efts were preserved throughout the period of study.

If this pattern of growth is representative of what occurs in a natural situation, then size at transformation will be of great importance in terms of growth to maturity and/or adult body size. A small metamorph is faced with two possible, but not mutually exclusive, alternatives:

1. It could mature the same season as a larger metamorph, sacrificing the greater reproductive success associated with large body size, but minimizing the risk of predation before sexual maturity that would be incurred by delaying maturity for another year.
2. It could spend longer growing so that at sexual maturity - when growth slows - it will be the same size as a larger metamorph. This would entail the advantages associated with large body size, but would suffer from the fact that it may take a year to make up the additional growth , which would expose the animal to another year of potential mortality prior to reproduction.

Berven (1982) recorded variable ages of maturity within a population of *Rana sylvatica*, and found that delaying sexual maturity lead to an increase in body size at maturity. Hence it should not be assumed that a hypothetical 'small eft' will necessarily end up with a reduced lifetime reproductive success.

Consider the model overleaf:

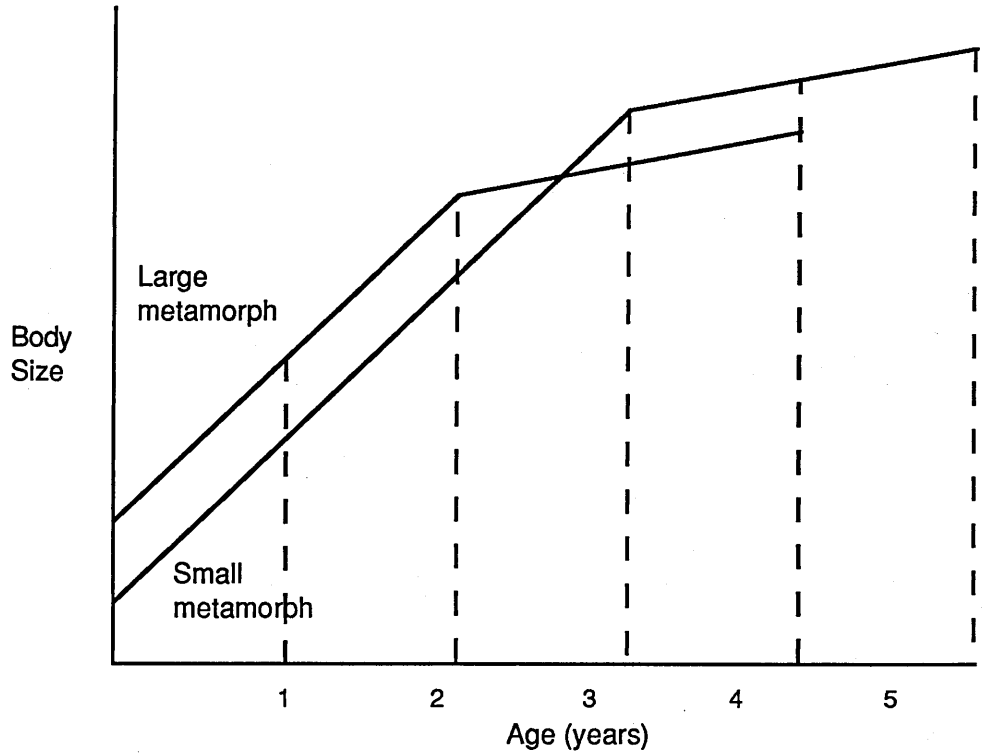


Fig. 8.2. Diagram to show alternative growth strategies for efts. Growth of hypothetical large and small efts is shown.

The above model shows that a small metamorph may take longer to attain maturity than a larger metamorph, but it may end up as a larger adult. As a larger adult, an individual (female) will have a greater annual fecundity.

The results of the present study are compatible with the results of Berven and Gill (1983), Smith (1987) and Semlitsch et al. (1988) that body size at transformation is related to body size at sexual maturity, in that the relative body sizes of metamorphs are preserved over the first ten weeks of eft life. Whether *Triturus vulgaris* maintains these differences in the field, until sexual maturity, remains untested. *T. vulgaris* does have a different life history pattern to the ambystomatids of the previously cited studies. Over 90% of growth in SVL of *Ambystoma talpoideum* occurs in the larval stage, and maturity is reached at one or two years. Hence any factor that affects metamorph size will be responsible for affecting a large fraction of the full growth potential. In *T. vulgaris* only 45% of adult

SVL is accounted for by growth in the larval stage (see Table 8.2 for calculation of this value). Hence factors affecting larval growth may have a weaker effect on adult body size.

Mean adult SVL = 46.0 mm (n = 105). See Section 1.2

Mean metamorph SVL = 20.7 mm (n = 16). Larvae reared at 20°C. See Chapter 4.

Therefore 45% of adult SVL is accounted for by larval stage growth.

Table 8.2 To show proportion of adult body size that is accounted for by larval growth in *T. vulgaris*.

Summary The growth of 28 *Triturus vulgaris* efts, maintained under constant, laboratory conditions, was recorded for the ten weeks immediately after metamorphosis. Mean increase in SVL was 21.6%. The relative body sizes of metamorphs were preserved over the period of study.

8.3 Growth of *Triturus cristatus* Efts After Transformation

Introduction This rearing experiment is similar to the one above in that efts were grown under captive conditions. However there are differences in the techniques used to rear the efts. Furthermore, prior to the study, larvae were all grown under identical conditions and the efts were reared to sexual maturity.

The study was intended to test two hypotheses. The first hypothesis is that body size at transformation is related to subsequent growth or size, particularly size at maturity. However, it has been found that *Triturus* larvae, grown under similar conditions, can show much variation in larval period, and those individuals that have longer larval periods also tend to have grown to a larger size (Chapter 4). So it is possible, that in *Triturus*, body size at transformation is not simply a fitness related trait, as suggested by Wilbur (1980), but also reflects the length of larval period, which is variable within a population. To investigate this possibility, body sizes of metamorphs were calculated relative to a value that changed with larval period to take into account the increase in body size with increasing time spent in the larval stage. This allowed a second hypothesis to be tested, that relative body size at transformation is related to subsequent growth or size, particularly size at maturity.

Method The efts used in this study were obtained from larvae reared in another experiment (see Chapter 4 and appendix), so that SVL, weight at transformation and length of larval period were known. After transformation 16 juvenile newts were housed in four plastic tanks (39 x 20 x 25 cm) filled with water to a depth of about 10 cm. A piece of expanded polystyrene was floated on the water surface to allow the newts to leave the water. However the animals remained aquatic for most of the time. The tanks were kept in a laboratory, so that water temperature fluctuated around 20°C. Photoperiod was not controlled, and all newts experienced approximately similar photoperiods throughout their juvenile lives. Newts were assigned to tanks on a random basis each time that the tanks

were cleaned out (every three weeks), in order to minimize differential tank effects or the effects of competition that may occur among captive newts (Spurway 1953).

The newts were fed on earthworms, pieces of beef heart, *Daphnia*, *Tubifex* and maggots (as sold to anglers). Food items were offered on six days a week, in quantities that would be eaten at once.

From 9-12-88 to 14-2-89 the newts were placed in a refrigerator to mimic a winter period of low temperatures. Newts were divided between three plastic boxes (28 x 15 x 10 cm) containing 2 litres of unconditioned tap water during this period. No food was provided during this period and weight loss was recorded. The newts were weighed after one week in the refrigerator, to allow emptying of material from the gut, and then weighed at the end of the wintering period, 60 days later.

Over their second growth season the newts were maintained in three tanks, kept either in a shed or within a tub as described in Chapter 3 for maintenance of adult smooth newts. Food was also only provided twice a week. Because of the decrease in rate of feeding and the different temperature regime experienced during the second growth season, comparisons between seasons cannot be made. However, rearing conditions did not vary between newts.

SVL was used as a measure of body size and efts were anaesthetized in MS-222 to allow measurement to the nearest 0.5 mm. Measurements were taken on the dates shown in Table 8.3.

Date	Mean days post transformation	
19-8-88	31	
7-9-88	50	
20-10-88	93	
22-11-88	126	
9-12-88	143	(Start of wintering)
14-2-89	210	(End of wintering - weighed only)
6-6-89	322	
20-10-89	458	Adult body size

Table 8.3. Dates of measurements of *T. cristatus* efts.

All newts appeared to be sexually mature by the end of the study. Males possessed tail stripes, large dark cloacas and small dorsal crests. Females possessed flat-topped, dome-shaped cloacas. Ten of the newts were female and six male.

Note that the efts used in this study commenced larval life, and transformed, at various times. Hence, the ages of newts on the dates of measurement, given above, represent the mean number of days spent in the eft stage.

Individual newts were identified by means of photocopy records of the pattern of yellow markings on the orange ventral surface, similar to the technique used by Hagström (1973) who used photographic records of newts to allow recognition of individuals in the field. The method used in the present study was as follows. Newts were anaesthetized by immersion in MS-222 (Sandoz), and placed on a sheet of transparent plastic. This was placed on a photocopier, covered with a sheet of white paper, and the newt was then photocopied. The belly patterns of juveniles are not fixed (Hagström 1973, Smith 1973), but photocopies of individuals during the course of this study confirmed Lantz's (1953) observations that the basic pattern of spots which is present at transformation remains constant, although the spots increase in size relative to the size of the animal. Provided that the interval between measurements is not too long, it is quite possible to identify individual juveniles.

Results To test the first hypothesis, that body size transformation is related to subsequent growth or size at maturity, Pearson product-moment correlation analyses were performed on: SVL at transformation and eft size, SVL at transformation and absolute growth (SVL increase per day), SVL at transformation and specific growth rate (SVL increase per day as a percentage of SVL at transformation). Correlation coefficients for data collected at seven ages post transformation are presented in Table 8.4.

Mean eft age (months)	Correlation coefficients for SVL at transformation and:		
	SVL	Absolute growth rate	Specific growth rate
31	0.87**	0.77**	0.63**
50	0.75**	0.54*	0.42
93	0.65**	0.39	0.18
126	0.77**	0.56*	0.31
143	0.74**	0.56*	0.33
322	0.79**	0.56*	0.33
458	0.63**	0.29	0.00

Table 8.4 Pearson product-moment correlation coefficients for size at transformation and size and growth rate to various ages of eft life of *T. cristatus*. * = Significant at 0.05 level, ** = significant at 0.01 level.

From Table 8.4 it can be seen that size at transformation was related to size at all other ages, including sexual maturity. Size at transformation was initially related to absolute growth, but this relationship decays with time. Size at transformation was not related to specific growth rate and the low correlation values here indicate that specific growth rate was independent of size at transformation.

To test the second hypothesis, that relative body size at transformation is related to

subsequent growth or size at maturity, a measure of relative body size was needed. Later metamorphs tend to be larger, so that body size differences between metamorphs may reflect time of transformation rather than growth during the larval stage. To compensate for this a regression of SVL on larval period was performed, and the residuals used as a measure of relative size, allowing for the tendency to increase in body size with length of larval period. See Fig. 8.3.

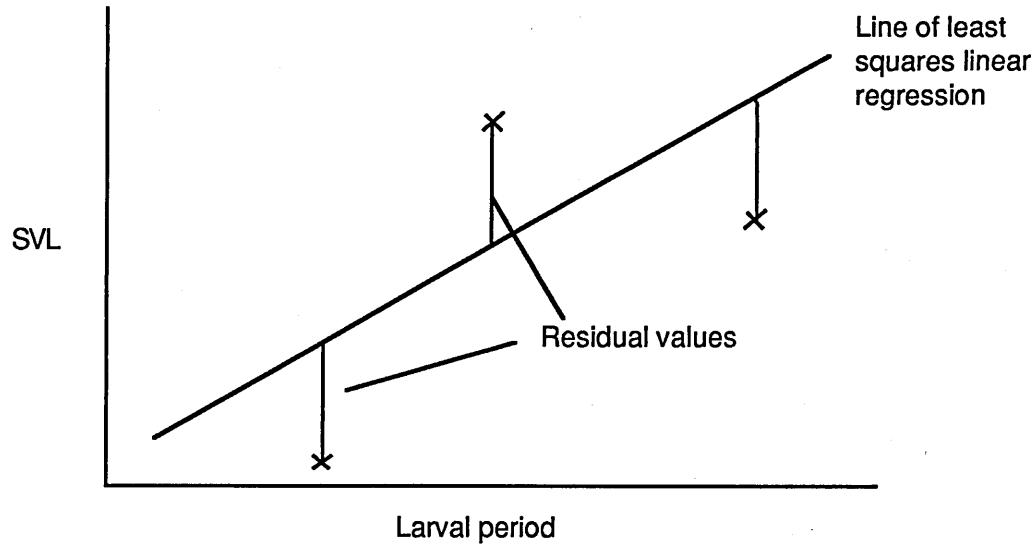


Fig. 8.3. To show calculation of relative body size.

The residuals obtained (relative body size) were then subjected to Pearson product-moment correlation analyses with SVL at transformation, absolute growth rate (SVL increase per day) and specific growth rate (SVL increase per day as a percentage of SVL at transformation). Correlation coefficient coefficients for data collected at seven ages post-transformation are presented in Table 8.4.

Mean eft age	Correlation coefficients for SVL at transformation and: SVL	Absolute growth rate	Specific growth rate
31	0.86**	0.39	0.31
50	0.72**	0.46	0.38
93	0.49	0.17	0.02
126	0.64**	0.38	0.20
143	0.59*	0.36	0.19
322	0.71*	0.56*	0.40
458	0.54*	0.30	0.09

Table 8.5 Pearson product-moment correlation coefficients for relative size at transformation and size and growth rate to various ages of eft life of *T. cristatus*. * = Significant at 0.05 level, ** = significant at 0.01 level.

From Table 8.5 it can be seen that relative body size is positively correlated with size at all measurement ages, including sexual maturity, but the relationship is not as strong as that between size at transformation and size at each measurement.

Since body size at transformation is related to size at sexual maturity, and it has been shown that large metamorphs tend to be those that have prolonged larval periods (Chapter 4.), it is possible that long larval periods are associated with large size at maturity. To test this hypothesis, a Pearson product-moment correlation coefficient was performed on larval period and snout-vent length at the seven ages at which measurements were taken, including size at maturity (see Table 8.6).

None of these correlations is significant (p-value at 5% level is 0.50) although there is a slight trend for larval period to be positively correlated with size at maturity.

Over the 60 days of the wintering period in a refrigerator, newts lost 11.5% of initial

body weight.

Mean eft age (months)	Correlation coefficients for larval period and SVL at later ages
31	0.38
50	0.37
93	0.45
126	0.47
143	0.47
322	0.43
458	0.37

Table 8.6 Pearson product-moment correlation coefficients for larval period and size at various ages of eft life of *T. cristatus*. * = Significant at 0.05 level, ** = significant at 0.01 level.

Discussion The results show that efts that are large at transformation tend to maintain their size advantage throughout the juvenile growth period and up to sexual maturity, so that large metamorphs mature as large adults. This is consistent with the field results obtained for other amphibians by Berven and Gill (1983), Smith (1987) and Semlitsch et al. (1988), so that it is possible that *T. cristatus* exhibits the same pattern of growth in the field.

The results of this study of growth in *T. cristatus* are also compatible with the results obtained from the growth of *T. vulgaris* efts earlier in this chapter. In the previous section, relative sizes of *T. vulgaris* efts were maintained over a ten week study period. *T. cristatus* also maintain their relative sizes during the period immediately post-transformation. However, the present study suggests that *T. cristatus* can also preserve

this ranking of sizes to maturity. It would be interesting to find out whether the same is true of *T. vulgaris*, but there are methodological problems associated with such a project. The present studies demonstrate the difficulties of maintaining *T. vulgaris* efts in a laboratory. Field enclosures might be a possible alternative. Enclosures built along the lines of outdoor 'reptiliaries' (see Leutscher 1961), that confine efts to a small area of terrestrial habitat can certainly support high densities of newts, with the benefit that they can obtain a reasonably natural diet (Leigh Gillett, pers. comm.). *T. alpestris* efts have been grown to sexual maturity in their second and third years in smaller versions of such outdoor enclosure (unpubl. data). However, the problem of marking *T. vulgaris* efts still remains, although *T. cristatus*, by merit of their individual belly patterns would be suitable for study in this manner.

It is also desirable to extend this study to more 'natural' growth in enclosures because growth in the field is slower than growth recorded in this present study. Newts in this study matured in the second year after transformation, which shows faster attainment of sexual maturity than is observed in wild populations (see Table 8.1). It is quite possible that the longer the timespan between transformation and maturity, the weaker is the relationship between body sizes at these two stages. This is also a relevant point to consider in evaluation of other studies of this pattern of growth. Semlitsch et al.'s *A. talpoideum* (1988) matured as one year olds and Smith's *Pseudacris triseriata* also matured rapidly, as one or two year olds. However, the fact that Berven and Gill's (1983) *Rana sylvatica* grew at a more typical rate for temperate species, and matured between one and four year olds is evidence that the positive relationship between size at transformation and size at maturity still exists among more slowly growing amphibians.

A potential fault of adopting a captive rearing programme in order to trace juvenile growth is that the high stocking densities, relative to the natural situation, may cause competition for food to occur as a laboratory-induced artifact. Spurway (1953) observed that captive *T. cristatus* established a 'social hierarchy'. However, no such behaviour was observed in the present study, and there was little increase in coefficient of variation in

body size to suggest that competition for food had occurred (coefficient of variation of SVL changed from 4.41 to 6.69 over the course of this rearing trial).

At this point it is necessary to reiterate some ideas expressed in Chapter 4. Chapter 4. shows that, in *Triturus* species, large body size at transformation is associated with a long larval period. However, extending the larval period incurs a risk, namely that it increases the likelihood of mortality due to pond desiccation. It was proposed that the benefit that counterbalanced this risk was that long larval periods allowed an individual to spend more time in a favourable growth environment. However, this hypothesis was not substantiated by the data presented in Chapter 4, since long larval periods were associated with slow overall larval growth. This is because long larval periods were achieved by extension of the shallowest part of the growth curve. The lack of evidence for any apparent advantage associated with long larval period can now be explained by results obtained from newts grown to sexual maturity in the present study. Although lengthening larval period may incur the risk of mortality due to pond desiccation and does not seem to provide a benefit from the advantages of extension of the rapid larval growth stage, results from the present chapter suggest that large size at transformation is maintained to maturity. Hence, although there were no significant correlations between length of larval period and body size of efts and adults, it may be of benefit to an individual to prolong larval period, since the increase in body size achieved may give it a size advantage over efts that transform earlier, at a smaller size.

The result that body size at transformation was more strongly correlated with size of efts and adults than was relative size at transformation, controlling for the effects of size increase with larval period, is also consistent with the scenario proposed above that prolongation of the larval period may be a strategy to increase size at sexual maturity. Individuals that transform at a large size relative to others with the same larval period will tend to be larger than these newts at maturity. However, the present data suggests that they are still unlikely to grow to a larger size than those individuals that attain a larger body size by prolongation of the larval period.

T. cristatus is sexually dimorphic in body size, females being larger than males

(Smith 1973). There were too few animals in the present study to reveal much information on how this difference is achieved in terms of juvenile growth. There was no tendency for females to be larger at transformation since the sexes were evenly divided between the eight smallest and largest metamorphs. The females tended to grow more during the eft stage. Female SVL increased by 22.9 mm compared to 20.25 mm in males, but this was not statistically significant ($t = 1.572$, $p = 0.138$, 14 d.f.). The growth of a larger sample of newts needs to be traced from transformation to maturity to establish the strength of this trend.

Conclusion Body size of *Triturus cristatus* efts at transformation was positively correlated with size throughout the eft stage and at sexual maturity. It is proposed that this size advantage at transformation may explain the variance observed in larval period of *Triturus* species. Long larval periods produce large metamorphs, which maintain their size advantage to maturity.

Chapter 9
Synthesis

This chapter summarizes the results of the work presented in this thesis and points towards future work that may answer some of the questions that have been raised during the course of this project.

9.1 Summary of work presented in this thesis Chapter 1 introduced the problem to be investigated. Adult body size in many species is determined by the amount of growth achieved during the pre-maturity stages. Adult body size is commonly positively correlated with reproductive success, an important determinant of fitness. Hence any factors that influence pre-reproductive growth will have important fitness consequences. The present project was conceived to identify and investigate the factors affecting pre-reproductive growth.

Chapter 2 reviewed the literature for evidence of body size effects on reproductive success in newts, with particular emphasis on *Triturus vulgaris*. In female smooth newts, body size is generally, but not unanimously, found to be positively associated with reproductive success, through the greater fecundity of large females. Among amphibian researchers it has become axiomatic that the positive association between female body size and fecundity implies that female body size restricts clutch size. The present thesis notes that there are other equally plausible explanations for this relationship (Section 2.1). The review of the relationship between body size and reproductive success in male smooth newts found that there is no direct or experimental evidence to support the hypothesis that large body size is correlated with high reproductive success.

Chapter 3 highlighted the need to further examine the nature of body size-fecundity relationships in female newts, and to test hypotheses concerning reproductive success and body size in male newts. Studies to investigate both of these areas are reported in Chapter 3.

In section 3.1 the effect of female body size on fecundity was investigated by counting the numbers of ova deposited by individual females of varying body size. This approach has not been adopted before due to methodological difficulties. These problems have been overcome during the course of the present study. The results of this investigation support previous assumptions that ovarian oocyte counts reflect annual female fecundity and also support the line of research that has found a positive association between female body size and fecundity.

Sections 3.2 and 3.3 tested the hypothesis that large male body size in smooth newts is positively associated with the ability to produce spermatophores. An anaesthetized female was used to test male spermatophore production, which removed the potential confounding effects of female motivation or mate choice on male performance. The results of these studies are that there is only a weak relationship between male body size and spermatophore production, with much variation in male performance, particularly among larger males. There was some evidence that male body condition may affect spermatophore supply in the short term, but this was not statistically significant. Male crest height was not correlated with the ability to produce spermatophores. Male body size had no effect on the length of time that a male could spend in courtship activity before rising to the surface to breathe.

Assuming that body size is heritable, then the higher fitness of larger females, within a population, may exert selection pressure for large body size (or whatever associated factors are responsible for increased fecundity) either in females alone, or in both sexes, if there is some genetic correlation between the sexes affecting body size. If large body size is associated with higher fitness, then the range of adult body sizes observed in natural populations of smooth newts (Chapter 1) warrants further investigation. Increasing body size with age was not sufficient to explain all variation in adult body size. This being so, then differential growth of individuals prior to sexual maturity must generate variable adult body size (Chapter 1). During the course of the present project three sources of variation in growth have emerged.

1. Intrinsic variation in rate of larval development (Chapter 4).
2. Environmental factors (Chapter 5).
3. Variation in timing of oviposition (Chapter 7).

1. Intrinsic variation in rate of larval development Chapter 4 showed that within a cohort of larvae, growing under similar conditions, there is little variation in the shape of the growth curve of individual larvae, but there is much variation in rate of differentiation. The result of this is that there is large variance in larval period, with consequent variation in body size of metamorphs. The rapidly developing larvae tend to be small at transformation, due to their brief time spent in the larval growth environment, whilst the more slowly developing larvae tend to be larger at transformation due to their longer occupation of the larval growth environment.

Large body size at transformation is associated with high fitness (Section 4.1), so it might be predicted that evolution should select larvae with longer larval periods, and hence large body size. However, there may well be costs associated with prolongation of the larval stage. Larvae with long larval periods are more prone to mortality due to pond desiccation or freezing than faster developing individuals. Newts breed in unpredictable and sometimes ephemeral environments, and so a bet-hedging strategy in terms of length of larval period may be the optimum life history strategy.

2. Environmental factors The effect of environmental factors on larval growth was examined. Chapter 8 showed that size at transformation was related to size at maturity, for newts grown under similar conditions, so for this discussion it will be assumed that deviations in body size of metamorphs, generated by environmental factors, will be translated into effects on adult body size. The next step in this line of research is to experimentally manipulate larval growth conditions and to determine whether effects on metamorph size are translated to adult body size.

Environmental variation affected two larval growth parameters; body size at

completion of the larval growth phase and length of the larval period. The effects of environmental variables on these two parameters are summarized in Table 9.1.

Environmental Variable	Larval parameter	mean	CV
Decreasing temperature	Body size	decreases*	same
	Larval period	increases	increases
Decreasing food supply	Body size	decreases	same
	Larval period	increases	increases
Increasing larval density	Body size	same/decreases**	same
	Larval period	increases/same***	increases

Table 9.1 Responses in metamorph body size and larval period generated by environmental variables. The above responses were recorded in *T. vulgaris* unless otherwise indicated. *Over most of temperature range. **Body size stays the same in *T. vulgaris* but decreases in *T. vittatus*. ***Larval period increases in *T. vulgaris*, but stays the same in *T. vittatus*. (CV = coefficient of variation).

From the results of the work described in Chapter 5, summarized in Table 9.1, it is apparent that changes in temperature, food supply and larval density affect body size at transformation, length of larval period and variation in these two parameters in broadly similar ways. Decreasing temperature or food supply and increasing larval density all may be regarded as changes in environmental variables that adversely affect larval growth. Body size at transformation is decreased and larval period is protracted. Variation in mean body size seems to be relatively unchanged, so that larvae appear to respond to less favourable growth conditions by increasing variation in larval period, rather than decreasing body size at transformation.

The changes in the two larval growth parameters, body size and larval period, under variation in environmental factors will probably have similar effects on adult body size. Small size at transformation is likely to be associated with small adult body size. Prolongation of the larval period, due to unfavourable growth conditions, is also likely to lead to small adult body size. A larva that transforms late in the year will not be able to accrue as much prey as an earlier transforming larva, in the eft stage, during the first year of life and will hence suffer lower growth. Prolonged larval periods are effectively equivalent to a slow initial growth season. Slow growth during this stage of life may result in reduced adult body size or an increased length of time spent growing to maturity.

Less favourable larval growth conditions did not tend to increase variation in body size at transformation. However, lowering temperature, decreasing food supply and increasing larval density all had the effect of increasing variation in larval period. Since less favourable growth conditions tended to increase variation in timing of transformation, they should also increase variation in adult body size, as described above.

In natural populations, environmental factors will probably affect adult body size in two ways; by generating variation in mean body size between years and between populations, and by generating variation around mean body size within year-classes. Variation in temperature, food level and larval density is likely to affect all larvae in one pond in a single year. For example, temperature or prey availability in larval ponds may behave in a stochastic manner. Hence, individuals within a pond may experience low mean temperatures or limited food intake, but this is likely to affect the whole population in a similar manner. A cold or low food year will produce small individuals at the end of the first growth season, and these may well mature as small adults, or simply take longer to mature than newts that experienced a good first year of growth. In situations where newts return to breed at their larval pond, variation in pond temperature and larval food supply will affect the size of adults in a year-class manner. So it would be expected that all individuals from a 'cold' or 'low food year' would either be smaller adults, or take longer to reach maturity than those from more favourable years. Individual newts can be expected

to have an adult life span that covers many seasons (4-5 in smooth newts, Verrell and Francillon 1986), so a breeding population may consist of individuals of many ages. The mean body size at maturity of each year class may be differentially affected by environmental factors such as temperature or larval food supply.

Variation in larval density between years will produce differences in mean body size of metamorphs and subsequent adults. Hence variation between year classes will be generated as described above for the effects of temperature and food level. Although larval density may not always behave in a stochastic manner (there is some evidence that larval amphibian populations may be regulated in a density-dependent manner [see Section 5.3]) its effect on adult body size will still be similar to the effect of varying temperature or prey levels.

There is evidence that metamorphic body size does vary between years, for the same breeding site. Semlitsch (1983) recorded significant differences in body size of metamorphic *A. tigrinum* between years, at the same pond. He was not able to attribute this to a specific cause, but proposed food supply, density and predation as possible causes.

In a situation where there is movement between breeding sites, and these differ in quality with respect to temperature, prey levels and larval density, it is quite possible that adult body size may be affected by the pond origin of an individual via the effects of these two factors. Savage (1952) noted large variation in the timing of transformation of *Rana temporaria* and *Bufo bufo* between spatially close ponds. He speculated that these differences may have been generated by different feeding opportunities within the ponds. Boisclair and Leggett (1989a) found that variation in prey consumption between populations of yellow perch (*Perca flavescens*) from different, but geographically close, lakes caused differential growth in the youngest age class of fish. Variation in density causing differential growth between spatially close populations has also been demonstrated in the same species (Boisclair and Leggett 1989b). Differences in prey levels and larval densities experienced by newt larvae from different ponds may generate similar between-

pond variance in growth. Eft dispersal to other ponds would cause mixing of newts of differing body size.

To what extent newts of the genus *Triturus* move between populations is unknown. However, Gill's (1978) work on *Notophthalmus viridescens* in America does suggest that newt populations may exist within a metapopulation-like structure, with the efts dispersing from their larval ponds to colonize nearby water bodies. If this is the case for *Triturus* species, then it is quite possible that variation in adult body size may be generated by migration of individuals between ponds of differing qualities and hence producing adults of differing body size. The implications of this are that not only do ponds differ in the number of newt larvae that are able to transform (as noted by Gill), but that these surviving metamorphs may also differ in terms of future lifetime reproductive success.

Adverse conditions for larval growth were shown to be able to generate increased variation in larval period in the laboratory (see Table 9.1). These results suggest that in the natural situation low temperatures or food supply or high larval density could all increase variation in timing of transformation, and hence adult body size within a year class, irrespective of migration between ponds.

3. Variation in timing of oviposition The prolonged period of oviposition of *Triturus* species (see Section 3.1) should contribute to size variation within a year class of adults. Although there was no size difference in metamorphs from early and late ova (Chapter 7) ova produced earlier in the year produce individuals that have a longer growth season than later ova. Hence these early cohorts may mature earlier or as larger adults than the newts from ova that were oviposited late in the year.

It is probable that growth during the eft stage is also affected by prey availability and temperature in much the same way as are larvae. Healy (1973) recorded varying annual growth rates for *Notophthalmus viridescens* efts between years and attributed this to differences in annual rainfall (efts were only visibly active when the weather was warm and wet). However, ecology of newts during the eft stage remains largely unknown.

Chapter 8 showed that the rank ordering of body sizes at transformation was preserved until maturity. It is suggested that in a large sample of newts, length of larval

period may be associated with size at maturity.

9.2 Areas for future work

1. Variance in male mating success. The factors affecting male mating success in newts still remain largely unknown. Sections 3.2 and 3.3 showed that there are no obvious features of male smooth newts that distinguish between males of differential mating capacity. Therefore measures of actual mating performance are still needed. One of the problems encountered in this line of research is that it is difficult to separate the effects of male parameters from female motivation (see Section 2.3). One solution may be to allow pairs of newts to court and record the spermatophore scores. Then, immediately afterwards, each male could be tested for further spermatophore production by use of a strait-jacketed female model as described in Sections 3.2 and 3.3. This would give two spermatophore scores per male. Total spermatophore production and spermatophore score in a courtship encounter. Total spermatophore score would represent male mating capacity, as in Sections 3.2 and 3.3, and the courtship encounter score would represent some measure of female motivation or mate preference.

2. Effect of weight condition and population density effects. In Section 3.2 a positive, but insignificant relationship was found between weight condition and spermatophore score. Weight condition has received little attention previously, even though it seems reasonable to assume that condition of individuals may affect growth and male mating success. Weight condition is easy to assess, using the technique described in Section 3.3. There may also be weight condition differences between populations. Veith (1987) found that weight condition of adult *T. alpestris* was inversely correlated with density in breeding ponds. Gill also found density effects in adults of *Notophthalmus viridescens*. So it is possible that density during the aquatic phase may affect individual weight condition.

3. In the area of female fecundity lines of further research may prove to be fruitful. The results of Section 3.1 suggest that female fecundity increases faster than does body size. A

repeat of my study, using a larger sample size would confirm whether this is indeed the case. The results of my study also showed that female clutch sizes can be slightly higher than previous work suggests. My largest females produced approximately 100 more ova than Verrell's (1986a) females. While the difference between maximum clutch sizes in the present study and other work is not great, it may suggest that female newts can actually yolk up ova during the aquatic phase, rather than just post-nuptially, as Harris (1987) suggests may be the case in *Notophthalmus viridescens dorsalis*. This possibility could be investigated by repeating my study of female oviposition, but using a control group of females that would have to be killed prior to oviposition to yield information on ovarian oocyte counts, to allow comparison with actual clutch counts of the ovipositing females.

4. Growth patterns of individual adults. Very little seems to be known of individual growth patterns of adults in species with indeterminate growth. For example, the observed relationship between female body size and fecundity has been traditionally treated as an advantage of large body size. However, it is equally possible that large female body size and high fecundity are both consequences of the same effect, namely that females may differ in absolute energy sequestered. The most obvious problem associated with the latter explanation is that if large female body size does not increase fecundity *per se*, then why do females not stay small and channel resources into reproduction? They would only grow large if there were other, non-fecundity related, advantages to large body size. These might include increased longevity, decreased metabolic demands and decreased rate of water loss per unit body weight. These advantages may outweigh the cost in reduced fecundity paid to attain large body size.

More precise data on growth patterns of individual adults may also yield information on resource allocation of individuals. For example, do smaller females grow more rapidly than large females? Do females that are small at maturity grow fast and catch up larger females? Does delaying maturity increase fecundity? In order to be able to answer such questions a between-season study of a species that breeds iteroparously, and allows a researcher a reasonable chance of being able to trace individuals between breeding seasons,

is needed. Crested newts (*Triturus cristatus* group) are a suitable study species, since adults return to the same breeding ponds each spring, are long-lived and individuals are recognisable due to unique belly patterning.

5. Field or large enclosure experiments to measure larval growth responses in a natural situation are still needed to establish the validity of *Triturus* larval growth responses that have been recorded in the laboratory in the present study. Density effects need particular investigation. The question of whether newt larvae show density-dependent growth in the natural situation has never been properly addressed. Previous work on *Triturus* species has assumed that larvae compete exploitatively for food, but with no evidence that this is in fact the case (Section 5.3). However, work on other urodeles, namely North American pond-breeding species, strongly suggests that under certain conditions, larvae can demonstrate density-dependent growth (Semlitsch 1983). This should provide the impetus to research the same area within the *Triturus* species. Such a study could benefit from the adoption of fisheries techniques of assessment of exploitative competition for food in fish, such as examining gut weight to body weight ratios between populations of different densities. Particularly intriguing is the possibility that adult populations may sometimes become food limited and hence compete with the larvae, as appears to be the case in *Notophthalmus viridescens* (Morin 1983). In a 'cattle trough' study, Morin found that the numbers of metamorphs emerging from each trough was negatively correlated with metamorph body size, and also with adult growth.

6. Little is known of the ecology or growth of newts during the eft stage. These subjects need further investigation, but are hampered by the problem that efts cannot be recaptured in large numbers. Hence the use of enclosures, as described in Section 8.3.4 may be one solution to this problem. The use of such a method may allow more rigorous testing of hypotheses concerning the relationship between body size at transformation and at maturity. The eft growth of metamorphs grown under differing larval conditions could be investigated in such enclosures, as could the growth of individuals grown under similar larval conditions that show differing size at transformation and timing of transformation.

Conclusion Variation in adult body size in newts occurs due to adaptive and proximate responses to occupation of an unpredictable larval environment. Life history strategies have evolved to cope with life in ponds that are unpredictable in quality and hydroperiod, and such strategies also generate variance in adult body size. For example, newts have evolved variable larval periods to exploit ponds of unpredictable quality and hydroperiod, and this results in variation in size of metamorphs and subsequent adults. Variation in larval growth conditions within breeding ponds also generates variation in adult body size. Biotic and abiotic factors such as larval density, prey levels and temperature may induce variation in mean and variance of resulting adult body size. The population dispersal mechanism, needed to colonize new ponds, also provides a potential mechanism for the mixing of adults of varying body size.

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